GENETICS

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BEARING ON HEREDITY AND VARIATION

Volume 21 JULY, 1936 Number 4

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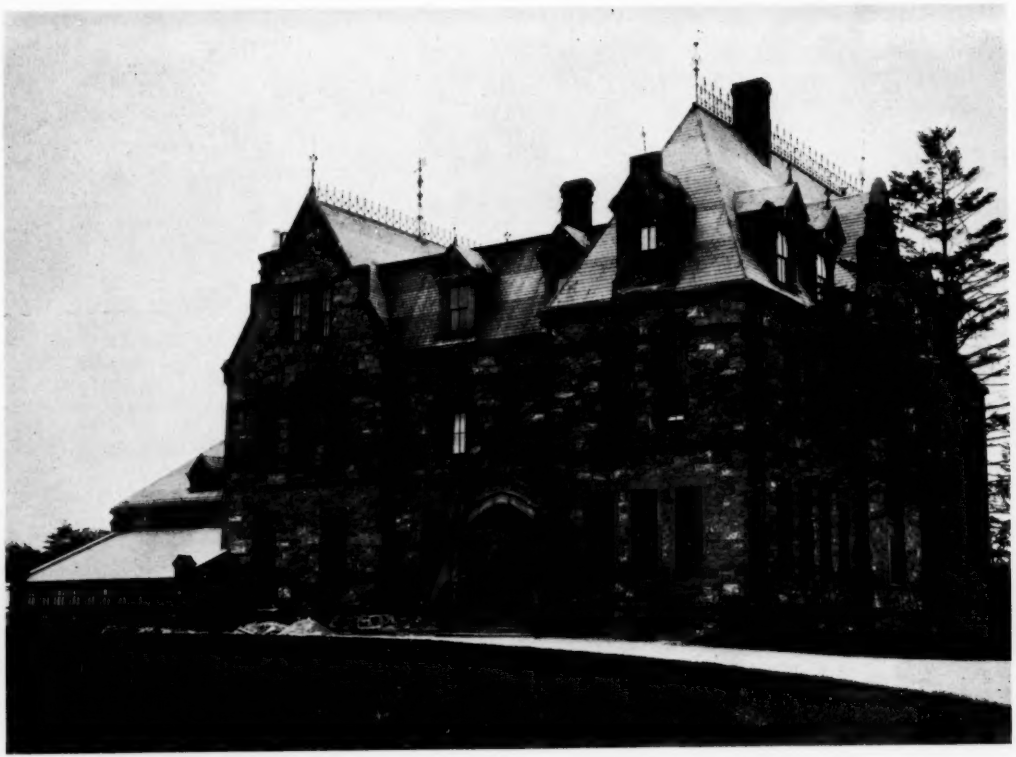

THE BUSSEY INSTITUTION OF HARVARD UNIVERSITY

Founded 1872—Closed June 30, 1936

THE Bussey Institution was founded in 1872, as a school of agriculture, and was named after BENJAMIN BUSSEY, on whose farm in Jamaica Plain the Institution was located. BENJAMIN BUSSEY'S will, drawn in 1835, provided for a school of agriculture and horticulture and anticipated, by more than twenty-five years, the Morrell Act of Congress, establishing the State Agricultural Colleges. The actual organization of the Bussey Institution was begun during the first year of President Eliot's administration (1869), and the building was completed in 1872.

The original faculty consisted of DEAN STORER, who taught agricultural chemistry; FRANCIS PARKMAN, as professor of horticulture; THOMAS MOTLEY, as instructor in farming; D. D. SLADE, as professor of applied zoology; and G. F. SANBORN, as instructor in entomology. The first contribution in plant breeding from the Bussey Institution was an article on "Hybridization of Lilies," published in the Bulletin of the Bussey Institution by FRANCIS PARKMAN, the famous historian.

PROFESSOR FARLOW began his work at the Bussey Institution in 1875, followed by PROFESSOR GOODALE and C. E. FAXON. FARLOW and GOODALE moved to Cambridge in 1879. In 1895 DR. THEOBALD SMITH was appointed to the professorship of applied zoology. He became affiliated with the Medical School and established the Antitoxin Laboratory, which was erected in 1903.

The Bussey Institution income was always meagre, but the early history is especially depressing. During 1879–80 DEAN STORER received $500, and several members of the staff received no salary. For many years the farm was used for boarding horses and cattle, and to provide vegetables for the students' dining hall at Harvard. The students paid for their tuition by cutting wood, which was sold to the professors in Cambridge.

The Bussey Institution functioned as an undergraduate school of agriculture for about thirty years, and was then reorganized as a graduate school in applied biology. PROFESSOR W. M. WHEELER was called from the Natural History Museum in New York to take charge of this work in 1908. The same year PROFESSOR W. E. CASTLE established his work in animal genetics at the Bussey Institution, and a year later PROFESSOR E. M. EAST was appointed to take charge of plant genetics. PROFESSOR C. T. BRUES joined the entomology department in 1910. In 1914 the department of forestry was affiliated with the Institution, and work in plant anatomy was started by PROFESSOR I. W. BAILEY. A year later economic botany, under the direction of PROFESSOR OAKES AMES, was included. PROFESSOR AMES

maintained his laboratory in the Bussey until 1926, when he moved to Cambridge as Curator of the Botanical Museum. In 1930 the Bussey faculty was merged with the faculty of arts and sciences, and the instructional and research work in entomology was transferred to Cambridge.

Research work in genetics and plant anatomy was continued at the Bussey for the next six years. The limited income made it necessary to close the Bussey in June, 1936. PROFESSOR CASTLE retired, after having served on the Harvard faculty since 1897, and the other members of the Bussey faculty were transferred to the new Biological Institute in Cambridge. The Bussey greenhouses and breeding plots have been retained for experimental work in plant genetics and cytology.

Since the reorganization of the Bussey as a graduate school, forty geneticists have received the degree of Doctor of Science, twenty in animal genetics and twenty in plant genetics. Nearly all these graduates are actively engaged in genetic research. Most of them hold important positions in various universities, federal bureaus, or agricultural experiment stations in the United States. A still greater number of geneticists have worked at the Bussey for shorter periods of time. Many of these men were postdoctorate students on traveling fellowships from foreign countries.

The Bussey Institution has given rise to three vigorous offspring: the Arnold Arboretum, the Antitoxin Laboratory of the State Board of Health, and, indirectly, the new Biological Institute. The work in genetics will be continued at the Biological Institute.

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STUDIES OF INHERITANCE IN LOP-EARED RABBITS

W. E. CASTLE AND S. C. REED

Bussey Institution, Harvard University

Received December 9, 1935

IN 1909 CASTLE published some observations on the behavior of ear length in crosses between lop-eared and ordinary varieties of rabbits, describing the inheritance as blending. Shortly thereafter LANG (1911) applied to these observations the multiple factor hypothesis which had been formulated by NILSSON-EHLE on the basis of his studies of quantitative characters in oats and wheat. Since then, numerous studies of quantitative characters in animals and plants have served to establish firmly the multiple factor hypothesis. But no further study has been made of the peculiar ear character found in lop-eared rabbits. CASTLE has meanwhile shown that among other varieties of rabbits there is a close correlation between general body size and ear length, skull length, and leg length. During development, the body as a whole and its various parts grow faster lengthwise than transversely. So the general body form, and that of all elongated organs, becomes more slender as the absolute size increases. Accordingly, other things being equal, the larger the rabbit, the longer proportionally are its ears, its skull, and its legs.

Lop-eared rabbits are one of the oldest recognized varieties of domestic rabbits and have undoubtedly attained their present excessive ear length as a consequence of long-continued selection. Other breeds of rabbits, for example Flemish Giants, exceed them in size of body, but none in length, width, and softness of the ears. This last characteristic (softness) they owe to a deficiency of cartilage in the ear, which makes it pliable and loppy and incapable of erection, as are the ears of ordinary rabbits. The body size of standard bred lop-eared rabbits is large, and that is one reason why their ears are long; but their ears are much longer than the ears in other breeds of rabbits of substantially the same body size. This shows that some additional agency operates to make the ears of lop-eared rabbits long. That it is a genetic agency there can be no doubt, for lop-eared rabbits are known to be true breeding, and their peculiar character in crosses is transmitted equally through male and female individuals in the blending manner generally recognized as indicating the operation of multiple genes.

In the light of what has been learned about heredity in the last highly fruitful quarter century, it has been thought desirable to make new observations on the results of crossing lop-eared rabbits with ordinary breeds of rabbits. To this end, in March 1932, a trio of purebred lop-eared rabbits was obtained from Mr. T. Corbishly, Secretary of the Lop-Eared

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Rabbit Club of England, through arrangements kindly made by Mr. E. C. Richardson.

The results to be described in this paper were obtained by (a) crossing the lop-eared male with females of other breeds; (b) raising an F_2 generation from F_1 parents; (c) backcrossing F_1 females to the lop-eared male; or (d) backcrossing F_1 individuals to the other parental (short-eared) stock.

METHODS AND STOCKS EMPLOYED

It has been our endeavor to grow the experimental rabbits under optimum conditions. To this end a mother has not been allowed to suckle more than 4 or 5 young at a time, the remainder of the litter, if any, being entrusted to foster mothers. The young have been weaned, as a rule, when one month of age. At that time each animal was weighed, and measurements were made of its ear length and ear width. Thereafter the animals were weighed and had their ears measured once every two weeks until they were four months old. At this age growth of the ears is nearing completion, although growth of the body may continue to the age of a year, or even longer.

As a basis for comparing individuals of different generations or litters, an estimate was made of the weight and ear measurement of each individual at the age of 120 days. Ear lengths were measured with a thin ruler from the base of the ear to its tip, the ear being held vertical and slightly stretched. Three or four independent readings were made at each measuring to minimize observational errors. At 120 days rabbits of small to medium body size will have practically completed their ear growth. The ears of rabbits of larger body size will continue to grow at a steadily diminishing rate for a few weeks longer, but the proportional change will be small and may safely be disregarded for statistical purposes. The lop-eared male was said to be about ten months old when we received him in March 1932. He then weighed about 4,000 grams, and his ear length was 25.5 cm. At four months old he probably had an ear length of about 24.0 cm, judging by the growth rates of our 3/4 blood lop rabbits. We shall accordingly rate him as a 24.0 cm rabbit.

He was mated with females of several different breeds, such as Blue Beveren, New Zealand Red, and the synthetic English-Dutch race of CASTLE. These range in body weight between 3,000 and 4,000 grams and in ear length from 11.0 to 13.5 cm. He was also mated with rabbits of smaller size, Himalayan and Polish derivatives. These ranged in weight from 2,000 to 3,000 grams and in ear length from 10.0 to 10.9 cm.

THE F_1 GENERATION

Eleven females in matings with the lop-eared male produced a total of 65 F_1 young, which were reared to maturity. The ear length of these in

TABLE 1

Ear length in cm of the F_1 animals at age 4 months in relation to ear length of their mothers

MATING	EAR LENGTH OF MOTHER	11.5	12	12.5	13	13.5	14	14.5	15	15.5	16	16.5	17	17.5	18	18.5	19	19.5	NO.	MEAN	S.D.	C.V.
A	10.0	1					1	2	3	1									8	14.57	1.17	8.0
B	10.2							1	6										7	15.15		
C	10.4							2	1		1								4	15.25		
D	10.4									2	2		1	1					6	16.48		
E	10.9								1	1			1	2					5	16.66		
	Total (A–E)	1					1	5	11	4	3		2	3					30	15.53	1.22	7.8
F	11.5										1		3	2					6	17.21		
G	11.8								1	1	1		1	1	1				6	16.70		
H	12.2										2	3	4	1					10	16.85		
I	12.0										1		1		1	1			4	17.50		
J	13.4												1	1	2				4	17.87		
K	13.5										1		1		1	1		1	5	17.98		
	Total (F–K)								1	1	6	3	11	5	5	2		1	35	17.23	.95	5.5

TABLE 2

Variation in ear length of the F_2 animals at age 4 months

MATING (SEE TABLE 1)	12.5	13	13.5	14	14.5	15	15.5	16	16.5	17	17.5	18	18.5	19	19.5	20	NO.	MEAN	S.D.	C.V.
♀A×♂A	1		2	1		2		3									9	14.61		
♀E×♂A				1	1			3				1					6	16.10		
♀D×♂D				1		1		1		1							4	15.67		
Total	1		2	3	1	3		7		1		1					19	15.16	1.33	8.8
♀♀C and E×♂H						2	4	5	1	3	3	1					19	16.48		
♀H×♂H			1	1	1	1	5	11	5	11	3	6	1	1			47	16.72		
♀♀I and J×♂H					1		1	3	2		2	5	1	1		1	17	17.46		
♀C×♂I						2	3	1	2	2	3	1	5	1			20	17.24		
♀E×♂I								3	1	2	2	5	1	1			15	17.64		
♀♀F–K×♂I							1	1	3	5	8	2	5	3			28	17.75		
Total			1	1	2	5	14	4	14	23	21	20	13	7		1	146	17.17	1.18	6.8

relation to that of their mothers is shown in table 1. In general, the greater the ear length of the mother, the greater the ear length of the offspring, all having the same father, the lop-eared male with an ear length of 25.5 cm when full grown. One F_1 individual was peculiar in having ears only slightly longer than those of its mother and not at all lopped. This animal (♀ 2994) at 4 months of age had an ear length of 11.6 cm, at which measurement it remained permanently. The mother of ♀ 2994 came of a purebred Himalayan family, and the seven sibs of the peculiar individual were very uniform in ear length (see table 1, Mating A); but this one aberrant individual had singularly short ears and transmitted that peculiarity to her offspring, as we shall see.

In general the ear length of the F_1 animals is nearer to that of the small races. This is not a matter involving superior maternal influence, since in a litter produced by the reciprocal cross (lop-eared ♀ × normal ♂) the F_1 young had similar ear length.

THE F_2 AND BACKCROSS POPULATIONS

An F_2 population was produced consisting of 165 individuals. Of these, 19 resulted from matings within the first group of F_1 individuals (matings A–E). Their variation in ear length is shown in the first section of table 2, the mean being 15.16 cm. The mean for the 146 F_2 individuals classified in the second section of table 2 is 17.17 cm. As compared with the 19 F_2 individuals of the first section of table 2, these are larger animals and have longer ears, as might be expected from their ancestry.

A backcross of F_1 females to the pure lop male (their father) produced a population of 68 animals which are listed in table 4 as 3/4 lop. Twenty-three of these, descended from a small-sized grandmother (matings A and E, table 1), had an average ear length of 19.20 cm. The remaining 45 were descended from larger mothers (matings F–H, table 1). They had an average ear length of 20.07 cm.

Backcrosses of F_1 animals to the maternal parent stock produced the 239 rabbits listed in table 4 as 1/4 lop. The peculiar F_1 female (2994) which had an ear length of only 11.6 cm, produced 18 backcross young. They form a compact group, varying closely about a mean of 10.98 cm, which is intermediate between the ear length of their mother (11.6 cm) and that of their Himalayan father (9.9 cm). The litter mate sisters of this female, likewise from mating A but having ears close to 15 cm long, produced 71 backcross young. They vary about an average of 12.09 cm, which again is intermediate between the ear lengths of the respective parents, 15 and 9.9 cm. The peculiar short ear character of ♀ 2994 would not appear to have been due to a single inhibiting or modifying factor derived from her mother, for in that case her backcross young should show a bimodal dis-

tribution owing to segregation on the part of the inhibiting factor. But in reality her backcross young are less variable than the young of her sisters (row 2, table 4), since their standard deviation was only .56 cm and their coefficient of variation 5.1; whereas her sisters' young had a S.D. of .76 cm and a C.V. of 6.2.

All backcrosses to the smaller Himalayan race, when combined, show an average ear length of 11.86 cm; all backcrosses to larger females show an average ear length of 14.42 cm. The coefficient of variation for the two groups is substantially the same (see table 4).

DISCUSSION

The F_1 young of small race mothers are more variable in ear length than the F_1 young of large race mothers, the former having a coefficient of variability of 7.8, whereas that of the latter group is 5.5. This indicates that the small mothers were less homogeneous than the large ones in regard to factors influencing ear length. The F_2 generation bears out this interpretation. The F_2 group from small mothers has a C.V. of 8.8, whereas the group from large mothers has a C.V. of 6.8. The backcross to the pure lop-eared male has a similar implication. The young descended from small mothers have a C.V. of 6.6; the corresponding group derived from large mothers has a C.V. of 5.7. CASTLE (1922) has shown that among ordinary (non-lop) rabbits a close correlation exists between body weight and ear length ($.836 \pm 0.011$). In the present investigation body weights and ear measurements were taken of 67 adult individuals as diverse in size as could be found in the laboratory at that time, ranging in weight from 1,400 to 4,700 grams. In this case also a fairly high correlation was found between body weight and ear length, namely, 0.76 ± 0.03. This indicates that among ordinary rabbits variation in ear length occurs chiefly as a consequence of variation in general body size. The genetic agencies which produce large body size automatically produce long ears and *vice versa*. But the case is very different with lop-eared rabbits. Here a body size of 4,300 grams, which among ordinary rabbits would be associated with an ear length of about 13.5 cm, is found associated with an ear length some 12 cm greater, an increase of 90 percent. We are concerned with investigating what genetic agency has caused this remarkable change.

It might be expected that so great a change in the size of the ear would be attended also with some change in its shape, but this apparently is not true. The relation of ear width to ear length remains about the same, irrespective of the absolute size of the ear. In the 67 adult individuals of various races, the ear length of which we measured, the ratio of ear length to ear width varied closely about a mode of 56 percent, regardless of absolute size (table 3). In a group of twelve 3/4 blood lop rabbits, the group,

of all those raised by us, having maximum ear size, the variation was very similar in character and the mean identical at .554.

TABLE 3

Ratio, ear length to ear width

	.51	.52	.53	.54	.55	.56	.57	.58	.59	.60	Mean
Frequency, mixed group	3	4	5	7	12	19	10	3	2	2	55.4
Frequency, 3/4 lop group			1	2	3	3	3				55.4

In the growth of the ear, as well as in its completed state, the relation of length to width changes very little from one month of age to maturity. Previous to one month, change in ear shape is more noticeable.

The twelve 3/4 blood lop individuals whose length-width ratios are recorded in table 3, gave the following average ratios at earlier ages.

Age in months	1	1 1/2	2	3	4	5	6
Mean ratio	59.03	58.03	57.60	57.10	56.32	56.34	55.91

The change in the shape ratio was small and gradual between the ages of 1 and 6 months, amounting in all to only 3 percent. If, instead of calculating such ratios, ear lengths are directly plotted against ear widths on a logarithmic scale for the period between one and six months of age, lines apparently perfectly straight are obtained. Both methods indicate that no genes affecting shape independently of size are in evidence during the period of maximum growth.

It would seem that all races of ordinary rabbits, irrespective of body size, would be equally available for crossing with pure lop-eared rabbits in a study of the genetic agencies responsible for the lop character, since all show a similar relation between body size and ear length. The genetic agency responsible for the added ear length of lop rabbits seems entirely lacking in ordinary rabbits, which differ in ear length chiefly because they differ in body size. If, accordingly, we can devise a means for equalizing the body weights of all rabbits studied, we can estimate what corresponding ear length each would be expected to have.

The regression of ear length on body weight was calculated for the 67 adult non-lop rabbits already mentioned. It was found that ear length increases one millimeter for each increase in weight of one hundred grams. The average body weight for the population of F_1 lop rabbits at four months of age was 2,400 grams. If all animals listed in table 4 had this same body weight, their ear lengths would be directly comparable. Accordingly an ear length was calculated for each individual on this basis, by use of a

TABLE 4

Actual ear lengths at four months of age of the various populations produced by crossing ordinary rabbits with a lop-eared individual

MATING	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15	15.5	16	16.5	17	17.5	18	18.5	19	19.5	20	20.5	21	21.5	22	NO.	MEAN	S.D.	C.V.
F_1, A–E				1					1	5	11	4	3		2	3										30	15.53	1.22	7.8
F_1, F–K											1	1	6	3	11	5	5	2		1						35	17.23	.95	5.5
F_2, A–E						1		2	3	1	3		7		1		1									19	15.16	1.33	8.8
F_2, C–K								1	1	2	5	14	22	14	22	19	19	14	7		1					146	17.17	1.18	6.8
1/4 Lop, A ♀ 2994	2	6	6	3	1																					18	10.98	.56	5.1
1/4 Lop, sisters of ♀ 2994		2	11	19	17	13	5	3		1																71	12.09	.76	6.2
1/4 Lop, F–K					2	3	15	29	34	26	17	15	7	2												150	14.42	.92	6.3
3/4 Lop, A–E														3	1	1			6	4	5	3				23	19.20	1.28	6.6
3/4 Lop, F–K														1			3	2	7	6	6	11	5	1	3	45	20.07	1.15	5.7

TABLE 5

Calculated ear lengths at four months of age of the populations of Table 4, if all had the same body weight, 2,400 grams

	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15	15.5	16	16.5	17	17.5	18	18.5	19	19.5	20	20.5	21	21.5	22	TOTAL	MEAN	S.D.	C.V.
P_1, Parents A–E		4	1																						5	11.10		
P_1, Parents F–K			1	1	1	2	1																		6	12.58		
F_1, Matings A–E			1						3	12	5	4	1	2	2										30	15.36	1.08	7.0
F_1, Matings F–K											2	4	7	9	7	5	1								35	16.99	.74	4.4
F_2, A–E					1			4	2		3	4	2	1	1	1									19	15.45	1.36	8.8
F_2, C–K								1	1	4	8	25	17	19	19	23	19	9		1					146	17.16	1.14	6.6
1/4 Lop, ♀ 2994, Mating A	3	9	5	1																					18	11.11	.39	3.5
1/4 Lop, sisters of ♀ 2994		3	10	17	19	14	5	2	1																71	12.42	.73	5.9
1/4 Lop, Matings F–K					4	9	20	41	27	25	12	10	2												150	14.41	.85	5.9
3/4 Lop, Matings A–E														2	2	1	1	3	5	9					23	19.13	1.01	5.3
3/4 Lop, Matings F–H														1		2	2	5	6	8	11	5	4	1	45	20.01	1.04	5.2

conversion factor of one mm for each hundred grams of weight. The observed ear length was increased if the animal weighed less than 2,400 grams and decreased if the animal weighed more than 2,400 grams. Thus were obtained the calculated results shown in table 5. As thus treated, the F_1 populations become less variable, and the difference between the progeny of small mothers (Matings A–E) and the progeny of large mothers (Matings F–K) becomes less. The former group still has the greater C.V. (5.5 as compared with 3.7), but this difference is due, principally, to the very aberrant individual produced in Mating A which had an actual ear length of 11.6 cm and a calculated ear length of 14.0 cm (table 5). Nevertheless, the mean and mode for the ear length of the F_1 progeny of small mothers remain lower than those for the F_1 progeny of larger mothers (Matings F–K). They differ by almost two centimeters. This indicates that the two groups differ by more than body size alone. The small mothers apparently carry certain genes which tend to make the ears short, irrespective of body size. But it may be that this apparent difference is due, in part at least, to the known earlier maturity of rabbits of racially small body size. The earlier the maturity of a rabbit, the more nearly would it have completed its growth at four months of age. The amount by which it falls below standard size would thus be *underestimated*, and the added ear length computed would fall below what it would be if all individuals had, at four months old, completed the same proportion of their full growth.

The F_2 populations show a relation similar to that shown by the F_1 populations. Those from small and short-eared mothers (Matings A–E) have ears over a centimeter shorter than those derived from Matings F–K. Both groups are more variable than the F_1 groups from which they were derived. The C.V. for the F_1 groups were 7.0 and 4.4 respectively; for the corresponding F_2 groups the values are 8.8 and 6.6 respectively. Note, however, that the variation in F_2, as well as in F_1, is slightly less when corrected or "equivalent" values are taken (table 5) than when uncorrected values are taken, as in table 4.

The 1/4 blood lop populations produced by backcrossing F_1 to the short-eared parent races, as calculated in table 5, cover the range between the ear length of ordinary rabbits (non lops) and that of the F_1 populations. There is still the same difference between the derivatives of Matings A–E and the derivatives of Matings F–K, the former being shorter in ear length by two centimeters, either because of the presence of special genes for short ear in the small races, or because their earlier maturity results in under correction, or for both reasons. The variability (C.V.) is the same in both groups of 1/4 blood lops (5.9). It is less than in the F_2 groups, but greater than in the F_1 groups, as we should expect on the multiple factor hypothesis.

Similar differences between the derivatives of Matings A–E and the derivatives of Matings F–K are found among the 3/4 blood lops (last two rows of table 5). The former are shorter eared by almost one centimeter. Their variability (C. V.) is substantially the same, but is somewhat less than that of the 1/4 blood lop populations. This again is what we should expect, as the lop-eared male which sired all the 3/4 blood lop individuals was purer, racially, than the mothers of the ½ blood lop populations. His race had been long selected for maximum ear length. The mothers in matings A–K and in the backcrosses which produced the ½ blood lop populations, were from races largely unselected as regards ear length. The equivalent ear lengths of the 3/4 blood lops do not quite cover the range between F_1 and the pure lop-eared ancestor.

On the whole, there is nothing in the results of these experiments at variance with the accepted multiple factor theory of inheritance, which supposes that an apparently blending inheritance results from the action of numerous genes located in many different chromosomes and so segregating independently.

However, we can not ignore the possibility that cytoplasmic, as well as gene, influences may be involved in producing an altered ratio between body weight and ear length, as, for example, in the peculiar short-eared F_1 female, 2994. She is similar in body size to her sibs, and yet has much shorter ears. It would seem, accordingly, that she must possess one or more genes which inhibit the action of the lop-ear genes inherited from her sire. If so, she should be more highly heterozygous for genes affecting ear length than her more normal sisters. For, like them, she would have inherited from their common sire (the purebred lop male) a full set of lop-ear genes, but from their mother she would have inherited *more* than their equipment of short-ear genes. Hence she would be more highly heterozygous than her sisters, and her gametic output would be potentially more variable. In reality, however, when mated to a purebred Himalayan male, she produced a group of 18 offspring which were *less variable* than the 71 offspring produced by her sisters mated to the same male.

A possible alternative explanation, in terms of genes, would be to suppose that a mutation had occurred in the gamete contributed by the lop-eared male to produce ♀ 2994, so that it carried less than the normal equipment of lop-ear genes. The zygote, in that case, (1) would have a lower (somatic) ear length than her sibs of identical parentage, and (2) would be less heterozygous than they for genes affecting ear length, as is indicated by the 1/4 lop population (table 4).

A more orthodox Mendelian explanation is equally possible, it being assumed that an extreme minus variant coming from the lop-eared parent happened to combine with an extreme minus variant from the short-eared

parent, the result being a zygote somatically extremely low in ear length but with only normal variability in the next generation about a lowered (intermediate) mean.

The blending or intermediate character of the inheritance of lop-ears, both in F_1 and in the backcrosses, shows that the genes which differentiate pure lop-eared rabbits from ordinary ones must be numerous and cumulative in their action. How numerous they are, it is impossible to say. Application of the CASTLE-WRIGHT formula (CASTLE 1921) to the F–K matings (table 6) indicates that the lop race differs from the F–K non-lop parental animals by about 22 genes for ear size, if it be assumed that each of these genes exercises the same quantitative influence as every other such gene. Since this assumption is of doubtful validity, the estimated number, 22, is probably too high.

A study of the range of variability of F_2 and backcross generations often allows one to estimate the number of important genes affecting the expression of a quantitative character. Possibly both parental types (short and lop-ears) would have been regained in the F_2, had a sufficiently large generation been raised. In the backcross to the parental race with short ears, 20 out of 150 of the backcross young had ears as short as those of the short-eared parents (table 4). The parental range was also regained when the F_1 animals were backcrossed to the lop race. Were there as many as 10 factor differences (all equal and additive in their action) between short- and lop-ears, we should expect to regain the parental type of factor combination in only one out of 1,024 animals. Actually we regained the parental type in about one out of every 10–50 animals, which allows us to conclude that the number of important factor pairs differentiating the lop race from ordinary rabbits is probably less than ten.

If genes located in particular chromosomes are responsible for the greater ear size of lop-eared rabbits, the question arises, is it possible to identify such chromosomes by means of linkage studies? We have a certain amount of data bearing on this question, but its indications are wholly negative.

1. In backcrosses to the short-eared Himalayan race employed in Mating A, there were produced 48 colored young having a mean ear length of 14.91 cm; also 34 Himalayans having a mean ear length of 14.60 cm, a non-significant difference indicating no linkage between short ears and albinism.

2. The dominant English coat pattern was introduced into certain of the F_1 individuals in Mating H. Among the 1/4 lop animals derived from this cross there were 79 English individuals having an estimated ear length of 16.78 cm, and 51 non-English with an average ear length of 16.62 cm, an insignificant difference.

3. The agouti factor was introduced into certain F_1 individuals by the non-lop parent. Among the 1/4 lop anmials derived from Matings F–K there were 34 agouti individuals with an average ear length of 16.86 cm, and 24 non-agoutis with an ear length of 16.73 cm, a difference devoid of significance.

4. The lop-eared male was a sooty yellow. All F_1 individuals which were black pigmented consequently carried yellow as a recessive character which should show coupling with lop-ear. Back-crosses of such F_1 black females with their father produced, among the 3/4 lops, 29 sooty yellows and 32 blacks. The former had an average ear length of 19.63 cm; the blacks had an ear length of 20.04 cm. There is thus seen to be no difference in ear length favoring the yellow young.

These findings, inconclusive though they are, on account of the small numbers observed, give no indication of the presence of genes for lop-ear in the four chromosomes which harbor the genes for albinism, English, agouti, and yellow, respectively. It is true that these are only four of the 22 chromosome pairs of the rabbit, and it is possible that genes affecting ear length are located in the 18 other chromosome pairs. It is also true that if a single gene for lop-ear were present in one of these chromosomes, yet had by itself only a minor influence on ear length, this influence might well be masked by the influence of other genes present in undetermined combinations.

We have seen that ear size is influenced by general body size, which, in turn, is influenced by rate of development of the fertilized egg. But there must be also in lop-eared rabbits an especial accelerated development in the head region, which makes the ears of lop-eared rabbits, or lop-eared crossbred derivatives, noticeably larger at birth than the ears of ordinary rabbits. The persistence of such an initial difference would account satisfactorily for the subsequent greater ear length of lop-eared rabbits. We can not assume that the supposed accelerated development in the head region is due to a special localized organization of the cytoplasm of the egg, since we know that the sperm is equally capable of inducing it.

The case of the short-eared mouse shows us that a single gene may be responsible for retarded development of the ear, which makes its ultimate length less. We know definitely that the gene in this case is located in the same chromosome with the gene for dilution. It seems reasonable to suppose that, in the case of the rabbit, a series of gene mutations having an opposite effect on ear length has taken place; that each of these, considered separately, has only a minor effect, but cumulatively they make all the difference between the lop-eared and ordinary rabbit. Yet the initial mutation or chance coincidence of mutations which formed the starting

point for human selection must have been sufficiently obvious to attract attention, after which new mutations having a like effect would be incorporated and retained in the selected race. There is reason to think that mutations having a contrary effect also occur among rabbits as well as among mice. The breeders' standard for Polish rabbits insists that the ears be as short as possible. This shortness is secured, in part, by keeping the body small, but to some extent also by special shortening genes. For it will be observed, in tables 4 and 5, that in our Mating A–E, in which mothers of small racial size were used, the progeny have shorter ears than the derivatives of mothers of larger racial size, even when correction is made for the differences in body size among the progeny. This indicates that in the rabbit, although there is (other things being equal) a strong correlation between general body size and ear size, there are also special genes influencing ear size, and chance mutations in these have been utilized in the production of lop-eared rabbits in large-bodied races and of notably short-eared rabbits in small-bodied races. This conclusion is in harmony with WRIGHT'S (1932) finding, based on a statistical examination of CASTLE'S size inheritance data, that although the various parts of the body form a harmonious system varying in unison and controlled by general body size, nevertheless to a minor extent variation in one part takes place independently of other parts.

SUMMARY

1. A renewed study of the inheritance of ear length in crosses between lop-eared and ordinary short-eared rabbits supports the multiple factor interpretation first applied to the case by LANG.

2. Ear length among ordinary breeds of rabbits is closely correlated with general body size. A similar influence of body size on ear length is found also among lop-eared rabbits, but special mutated genes in the case of purebred lop-eared rabbits nearly double the ear length as compared with that of ordinary rabbits of like body size.

3. The increased size of the ears in lop-eared rabbits has not been attended by any change in the shape of the ear. The ratio of ear length to ear width is nearly the same in all breeds of rabbits and at all ages between one month and maturity. It varies closely about a modal value of 0.56.

4. There are indications that mutations decreasing the size of the ears in relation to that of the body have also occurred among rabbits, and that such mutations have been incorporated in such small-bodied and short-eared breeds as Polish.

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STUDIES OF A SIZE CROSS IN MICE, II

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SEVERAL years ago GREEN made a cross between two races of mice of very different body size, the experiment being designed to throw light on the mechanism of size inheritance. His working hypothesis was that differences in body size are determined by genes borne in chromosomes, that such genes are numerous, and that accordingly it should be possible to demonstrate the existence of certain of them in the same chromosomes as contain coat color genes.

As a small parent race, he used *Mus bactrianus*; as a large race of nearly twice as great body size, he used LITTLE's dilute brown race of *Mus musculus*. The small race contained the three independently inherited dominant genes, agouti (A^w), black, and dilution. The large race contained the three recessive alleles, non-agouti, brown, and dilution. In F_2 and backcross populations it was found that larger body size was associated with each of the recessive segregates, non-agouti individuals being larger than agoutis, browns larger than blacks, and dilutes larger than intense individuals. This was regarded as supporting the hypothesis that in each of the three marked chromosomes derived from the larger parent there was present one or more genes for larger body size. On further investigation, however, GREEN concluded that the differences observed were sufficient to have unmistakable statistical significance only in the case of the chromosome containing the brown gene. This was the state of the question when we undertook its further investigation. We attempted a substantial repetition of GREEN's experiment with slightly different stocks available to us. As a small parent race we used at first not *Mus bactrianus* but a supposed domestic derivative of it, the race of black-and-white Japanese waltzing mouse studied by GATES (1926). Later, through the kindness of Drs. GREEN and LITTLE, we were able to employ also the same strain of *Mus bactrianus* which GREEN had employed. As the large parent race in both these crosses (which, for convenience, we may call Cross 1 and Cross 2, respectively), we used a derivative of LITTLE's dilute brown race in which two additional recessive gene mutations had been incorporated, namely pink-eye and short-ear.

The F_1 animals produced by both crosses were only a little smaller than animals of the larger parent race but were of remarkable vigor, fecundity, and longevity. Both were reciprocally backcrossed to LITTLE's dilute

brown race of *M. musculus*. The F_1 animals from Cross 1 were also backcrossed to the actual *musculus* parent race, the pink-eyed short-eared dilute brown race of GATES. We may now proceed to the discussion of these backcross populations.

BACKCROSS OF F_1 FEMALES FROM CROSS 1 TO D BR MALES

In a previous paper, we have discussed the mature body weight and the body length at age six months of this population consisting of 1,236 animals. We have shown, in confirmation of GREEN'S earlier conclusion, that brown individuals are heavier and longer bodied than blacks, and that dilute individuals are heavier and longer-bodied than intense ones, though the difference is less in the case of dilution than in that of brown, as GREEN had previously observed. But we have been led to adopt an explanation for the manifest superior size of brown and of dilute segregates different from that of GREEN. Instead of invoking the action of size genes located in the same chromosomes as the brown and dilution genes, we assume that it is the physiological action on growth of the brown gene and the dilution gene themselves which produces the observed effects. To this question we shall return later. For a detailed statement of results as regards weight and body-length, the reader is referred to our previous publication. CASTLE, GATES and REED (1936).

At the time these mice were chloroformed and measured as to body length, their tail length was also measured by SUMNER'S method, keeping the body under a uniform tension of 20 grams. We have only recently studied the data on tail length, which show one surprising and unexpected feature. The greater size of brown and of dilute individuals, as compared with black and intense individuals respectively, finds expression as expected in all three criteria studied, namely, (1) maximum weight at or prior to six months of age, (2) body length, and (3) tail length at age six months. Brown has regularly a greater influence than dilution on weight and body-length, as reported in our previous publication, but as regards tail-length their relations are reversed. Dilution has a greater influence than brown in elongating the tail. This is the unexpected feature of a study of tail length in the backcross population, and it finds support, as we shall see, in the backcross from Cross 2. The data on the variation in tail length of the backcross population are contained in table 1. The numbers there reported (637 females and 439 males) are smaller than for the body length studies reported in our previous paper because of occasional injury to the tail (particularly in the case of males caged together). A single domineering male will, by biting the tails of his cage mates, destroy the possibility of obtaining normal tail measurements for them. Nevertheless the available data for both sexes are entirely in harmony, in that they

show that the tail length of blues is greater than that of browns, the order of size among the color classes as regards tail length being (1) black, (2) brown, (3) blue, and (4) dilute brown. Brown females as compared with blacks have an increased tail length of 1.5 percent, but dilute females as compared with intense have tails 2.3 percent longer. Also brown males

TABLE 1

Variation in tail length of four different color classes from matings of F_1 females from Cross 1 to d br males

	FEMALES			MALES		
	NO.	MEAN	S.D.	NO.	MEAN	S. D.
Black	151	75.5±.22	3.98±.15	103	78.7±.27	4.09±.19
Blue	170	77.5±.19	3.76±.14	116	80.1±.24	3.86±.17
Brown	159	76.9±.22	4.08±.15	121	79.0±.27	4.40±.19
Dil. Brown	157	78.5±.22	4.07±.15	99	81.7±.23	3.38±.17
Total	**637**	**77.2±.11**	**4.10±.08**	**439**	**79.8±.13**	**4.15±.09**
All Blacks	321	76.5±.15	3.96±.10	219	79.4±.18	4.03±.13
All Browns	316	77.7±.16	4.18±.12	220	80.2±.19	4.20±.13
All Intense	310	76.2±.15	4.07±.12	224	78.8±.18	4.10±.13
All Dilute	327	78.0±.15	3.93±.10	215	80.84±.17	3.74±.12
Brown minus Black		1.20±.22=5.4			.80±.26=3.0	
Dilute minus Intense		1.77±.21=8.4			1.98±.24=8.2	

as compared with blacks, have tails 1.2 percent longer, but dilute males as compared with intense have tails 2.6 percent longer. In both sexes dilution has an influence on tail length superior to that of brown, although in regard to body length and total weight the relation is reversed. Both mutant genes (brown and dilution) for which segregation is occurring in this backcross population have a positive (increasing) effect on general body size, as indicated in body weight, body length, and tail length. Their combined action is also additive or cumulative in all cases. But apart from general body size, there would seem to be also a special specific influence of dilution on tail length.

BACKCROSS OF F_1 MALES FROM CROSS 1 TO D BR FEMALES

We were able to obtain a smaller population from this backcross than from the reciprocal one already discussed because of the smaller number of mothers available. The number studied is approximately 100 of either sex, more exactly 99 females and 106 males. Comparative data on the segregation in this and the reciprocal backcross are contained in table 2.

In making this comparison we are interested in two questions: (1) will the chromosomes marked by brown and dilution here show the same accelerating influence on growth as in the reciprocal backcross, and (2) is there a significant difference in size between the progeny of F_1 mothers

TABLE 2

Comparison of the several classes of individuals produced by reciprocal backcrosses between F_1 animals and the "d br" race

	FROM ♀F_1×♂*d br*				FROM ♀*d br*×♂F_1				DIFFERENCE		
FEMALES	NO.	MEAN WT. GMS.	MEAN BODY MM.	MEAN TAIL MM.	NO.	MEAN WT. GMS.	MEAN BODY MM.	MEAN TAIL MM.	WEIGHT GMS.	BODY MM.	TAIL MM.
Black	337	21.91±.08	91.51±.12	76.5 ±.15	41	23.18±.29	93.51±.34	76.82±.51	1.27±.30	2.00±.36	.32±.53
Brown	328	22.61±.12	92.84±.12	77.7 ±.16	58	23.73±.25	94.71±.27	77.27±.36	1.12±.27	1.87±.29	−.53±.39
Intense	324	22.04±.09	91.79±.12	76.2 ±.15	49	22.75±.24	92.88±.23	75.72±.44	.71±.25	1.09±.26	−.48±.46
Dilute	351	22.45±.10	92.55±.12	78.0 ±.15	50	24.24±.28	95.52±.30	78.27±.39	1.79±.29	2.97±.32	.27±.41
Totals and means	**675**	**22.24±.10**	**92.17±.12**	**77.1 ±.15**	**99**	**23.93±.25**	**94.18±.25**	**77.0 ±.40**	**1.69±.27**	**2.01±.28**	**−.01±.42**
MALES											
Black	280	28.34±.13	95.77±.14	79.4 ±.18	62	28.28±.21	97.14±.22	78.85±.39	.15±.24	1.37±.27	−.55±.43
Brown	291	29.56±.12	97.73±.10	80.2 ±.19	44	29.63±.29	98.48±.29	79.71±.51	.07±.27	.65±.30	−.49±.54
Intense	278	28.67±.12	96.26±.14	78.8 ±.18	53	28.51±.25	97.21±.24	77.58±.47	−.49±.27	.95±.27	−.22±.50
Dilute	293	29.34±.13	97.21±.14	80.84±.17	53	29.17±.23	98.24±.27	80.64±.32	−.17±.26	1.03±.30	−.20±.36
Totals and means	**571**	**29.00±.12**	**96.74±.13**	**79.82±.17**	**106**	**28.95±.24**	**97.76±.25**	**79.25±.35**	**−.05±.26**	**1.02±.28**	**−.47±.39**

mated with d br males and the progeny of d br females mated with F_1 males? In other words, has the large race mother any superior influence on the size of her offspring? Table 2 contains the answer to the first question, an emphatic affirmative. Brown and dilution are found in heavier and longer-bodied individuals than their alleles in this as in the reciprocal backcross. Also brown has a greater influence than dilution in both sexes in increasing the average weight and body length; but brown has in both sexes *less* influence than dilution in increasing length of the tail, exactly as in the reciprocal backcross. We may accordingly, for qualitative effects, combine the data from both backcrosses, weighting each in proportion to the number of individuals which it contains. This procedure gives us the weighted means printed in italics in table 3.

TABLE 3

Percentage change in body size effected by the gene mutations brown (b) and dilution (d) in reciprocal backcrosses between F_1 hybrids and the d br race

		FEMALES			MALES		
GENE	MOTHER	WEIGHT	BODY LENGTH	TAIL LENGTH	WEIGHT	BODY LENGTH	TAIL LENGTH
b	♀ F_1	3.18	1.45	1.56	4.30	2.04	1.20
b	♀ d br	2.37	1.28	.58	4.77	1.38	1.09
Weighted mean		*3.07*	*1.40*	*1.43*	*4.37*	*1.90*	*1.18*
d	♀ F_1	1.86	.82	2.36	2.30	.98	2.60
d	♀ d br	6.54	2.84	3.36	2.31	1.06	3.94
Weighted mean		*2.47*	*1.08*	*2.48*	*2.30*	*.99*	*2.80*

The second question, one of considerable theoretical interest, as it involves a possible differential maternal influence on the size of offspring, finds its answer in table 2. The d br (large race) mothers produce larger-bodied offspring in both sexes, if we rely on the criterion of body length alone, undoubtedly the most reliable criterion. The differences between the means of the two groups are statistically significant. If we judge by body weight, female offspring of the large race mothers are also significantly larger, but there is no significant difference in the case of males. As regards tail length, no significant difference is found in either sex.

RECIPROCAL BACKCROSSES OF F_1 ANIMALS FROM CROSS 2 TO THE D BR RACE

The F_1 animals from this cross showed (but were heterozygous for) the three dominant characters, agouti, black, and intensity. On backcrossing them with the d br race which is homozygous for the corresponding recessive alleles, non-agouti, brown, and dilution, eight classes of colored offspring were obtained. In the left half of table 4 and of table 5 will be found a summary of the data on the body size of each of these eight classes

TABLE 4

Comparison of the several classes of females produced by reciprocal backcrosses between F_1 individuals from Cross 2 and the d br race

FEMALES	FROM ♀ F_1×♂ *d br*				FROM ♀ *d br*×F_1				DIFFERENCE		
	NO.	MEAN WEIGHT	MEAN BODY	MEAN TAIL	NO.	MEAN WEIGHT	MEAN BODY	MEAN TAIL	WEIGHT	BODY	TAIL
Black Agouti	56	22.99	92.12	73.55	20	23.37	93.40	72.65	.38	1.28	−.90
Blue Agouti	62	23.48	92.67	74.47	13	24.01	94.30	75.40	.53	1.63	.93
Brown Agouti	60	23.97	93.76	75.42	16	25.55	95.06	75.33	1.58	1.24	−.09
Dil. Br. Agouti	45	24.42	94.72	77.17	17	24.82	94.94	75.79	.40	.22	−1.38
Black	56	23.30	92.75	74.41	16	24.05	94.50	76.35	.75	1.75	1.94
Blue	62	23.74	93.66	76.65	15	25.33	94.33	76.25	1.59	.67	−.40
Brown	54	24.75	93.37	74.44	15	26.28	95.00	74.59	1.53	1.63	.15
Dil. Br.	47	24.52	94.51	77.25	12	25.42	94.09	77.12	.90	−.42	−.13
Totals and means	**442**	**23.91±.09**	**93.38±.10**	**75.42±.13**	**124**	**24.85**	**94.45**	**75.30±.26**	**.96**	**1.01**	**.01**

TABLE 5

Comparison of the several classes of males produced by reciprocal backcrosses between F_1 individuals from Cross 2 and the d br race

MALES	FROM ♀ F_1×♂ *d br*				FROM ♀ *d br*×♂ F_1				DIFFERENCE		
	NO.	MEAN WEIGHT	MEAN BODY	MEAN TAIL	NO.	MEAN WEIGHT	MEAN BODY	MEAN TAIL	WEIGHT	BODY	TAIL
Black Agouti	56	28.79	96.24	75.69	19	29.06	97.10	74.87	.27	.86	−.82
Blue Agouti	52	29.80	98.18	78.25	14	31.92	99.71	78.82	2.12	1.53	.57
Brown Agouti	63	31.27	99.45	76.59	22	32.48	99.05	78.11	1.21	−.40	1.52
Dil. Br. Agouti	61	30.76	98.52	79.19	16	32.86	98.68	79.85	2.10	.16	.66
Black	54	29.31	96.00	75.37	16	29.78	97.18	75.83	.37	1.18	.46
Blue	51	30.48	97.74	76.98	15	31.12	99.06	78.57	.64	1.32	1.59
Brown	51	30.87	98.34	77.63	12	32.54	98.41	77.41	1.67	.07	−.22
Dil. Br.	55	30.47	97.93	78.63	19	32.50	100.03	79.72	2.03	2.10	1.09
Totals and means	**443**	**30.40±.11**	**97.83±.11**	**77.27±.15**	**133**	**31.53**	**98.65**	**77.78±.30**	**1.31**	**.85**	**.51±.33**

as indicated by weight, body length, and tail length, when an F_1 female was the mother. In the right half of these same tables will be found corresponding data for the reciprocal backcross in which a d br female was the mother.

TABLE 6

Percentage change in the body size of females effected by the gene mutations, a, b, and d, in reciprocal backcrosses between F_1 individuals from Cross 2 and the d br race

GENE	MOTHER	WEIGHT		BODY LENGTH		TAIL LENGTH	
		DIFF. AND P.E.	PERCENT INCREASE	DIFF. AND P.E.	PERCENT INCREASE	DIFF. AND P.E.	PERCENT INCREASE
	♀ F_1						
a	Bact.	.46 ± .14	1.94	.21 ± .20	.33	.68 ± .28	.84
a	♀ d br	.83	3.40	.05	.05	1.38 ± .52	1.84
Weighted mean		*.54*	*2.26*	*.17*	*.26*	*.83*	*1.06*
	♀ F_1						
b	Bact.	1.40 ± .18	1.94	1.21 ± .20	1.30	.68 ± .28	.84
b	♀ d br	1.33	5.48	.64	.68	.71 ± .52	.94
Weighted mean		*1.38*	*2.71*	*1.08*	*1.16*	*.68*	*.85*
d		.13 ± .17	.54	.77 ± .20	.82	1.77 ± .25	2.37
d	♀ d br	.08	.33	−.07	−.07	1.52 ± .48	2.04
Weighted mean		*.12*	*.49*	*.58*	*.62*	*1.71*	*2.29*

TABLE 7

Percentage change in the body size of males effected by the gene mutations, a, b, and d, in reciprocal backcrosses between F_1 inidividuals from Cross 2 and the d br race

GENE	MOTHER	WEIGHT		BODY LENGTH		TAIL LENGTH	
		DIFF. AND P.E.	PERCENT INCREASE	DIFF. AND P.E.	PERCENT INCREASE	DIFF. AND P.E.	PERCENT INCREASE
	♀ F_1						
a	Bact.	−.29 ± .21	−1.40	−.81 ± .22	−.83	−.23 ± .29	−.30
a	♀ d br	−.14	−.44	.03	.03	.59 ± .56	.76
Weighted mean		*−.25*	*−1.18*	*−.60*	*−.61*	*−.04*	*−.05*
	♀ F_1						
b	Bact.	1.30 ± .21	4.40	1.56 ± .20	1.60	1.50 ± .30	1.96
b	♀ d br	2.12	6.97	.78	.79	2.14 ± .58	2.79
Weighted mean		*1.49*	*4.99*	*1.37*	*1.41*	*1.64*	*2.13*
	♀ F_1						
d	Bact.	.32 ± .22	1.00	.74 ± .22	.75	2.10 ± .30	2.75
d	♀ d br	1.13	3.66	1.43	1.46	3.03 ± .57	3.97
Weighted mean		*.51*	*1.61*	*.89*	*.91*	*2.31*	*3.03*

The question as to the relative influence of F_1 mothers and d br mothers on the size of their offspring will be clear from an examination of these tables. In each of the eight color classes, in both sexes, the d br mothers produce the heavier offspring. The same superiority of the progeny of d br mothers is found as regards body length in seven of the eight color classes of each sex, and of course emphatically when all color classes are combined.

As regards tail length, there is no uniform superiority of the progeny of large race mothers, though in general, particularly among the males, d br mothers produce the longer-tailed progeny.

The relative influence of each of the recessive genes on the body size of the offspring in these backcrosses is shown in tables 6 and 7. Non-agouti females are larger than agouti females by all three criteria, weight, body length, and tail length (table 6), though as regards body length (the best criterion) the difference is negligible. But among the males a contrary relation is found, since agouti individuals are larger-bodied than non-agoutis by all three criteria. It is accordingly not shown that the non-agouti mutation, though derived from the larger parent race, has any consistent influence either to increase or to decrease body size.

The brown mutation (*b*) is shown in this cross, as in all others, to increase body size, as judged by all three criteria. Brown females are more than 2.5 percent heavier and brown males are 5 percent heavier than black individuals of the same sex. Brown females are also 1.1 percent longer bodied and brown males are 1.4 percent longer-bodied than black ones. Brown females have tails less than 1 percent longer than blacks, but brown males have tails more than 2 percent longer than blacks. By all criteria males show a greater increase in size than females, as in the backcrosses previously discussed.

Dilution, as in the other crosses, increases size in both sexes in lesser amount than brown as regards weight and body length, but in greater amount as regards tail length. Tail length is increased 2.29 percent in dilute females and 3.03 percent in dilute males, the corresponding increases for brown individuals being .85 percent and 2.13 percent respectively. A special influence of the dilute gene (or the dilute chromosome) on tail length is thus shown to occur in four backcross populations, namely, in the reciprocal backcrosses both from Cross 1 and from Cross 2.

BACKCROSS OF F_1 FEMALES FROM CROSS 1 TO MALES OF THE MATERNAL (SE) RACE

This backcross, like those already described, was made reciprocally, but owing to an insufficient supply of se females being available, only the backcross in which F_1 mothers were used has as yet produced a population large enough for useful discussion. We shall accordingly, for the present, confine our attention to this alone.

This backcross is of interest not only because it gives additional evidence on the effect of brown and dilution on body size but also because it yields data on the effects of two additional gene mutations, pink-eye and short-ear. Since short-ear is closely linked with dilution, the number of phenotypes produced by the backcross is eight, except when a crossover occurs

(about once in a thousand). The number of individuals produced was larger in this than in any of our other backcrosses. We present data on a population of 930 females and 917 males, a total of 1,847 individuals. It would be a needless use of space to report the detailed study which has been made of the variation of each color class in weight, body length, and tail length.

TABLE 8

Comparative variability of females of the several color classes produced by a backcross of F_1 females to the se race

		WEIGHT		BODY LENGTH		TAIL LENGTH	
	NO.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
Black	126	23.12±.18	2.95±.12	91.00±.20	3.25±.14	79.20±.26	4.13±.18
Blue se	133	22.82±.15	2.66±.11	90.27±.16	2.73±.11	78.77±.23	3.96±.16
Brown	124	22.71±.16	2.65±.11	90.59±.20	3.30±.13	79.32±.28	4.62±.19
D br se	124	22.49±.17	2.77±.12	90.92±.19	3.20±.14	78.29±.27	4.55±.20
PE Black	118	23.21±.17	2.81±.12	90.32±.20	3.21±.14	78.12±.25	3.90±.17
PE Blue se	98	22.02±.16	2.44±.12	89.93±.25	3.71±.18	78.13±.29	4.22±.20
PE Brown	119	24.09±.20	3.31±.14	91.42±.20	3.34±.15	78.91±.23	3.84±.16
PE d br se	88	22.50±.19	2.65±.13	90.76±.19	2.99±.15	78.03±.30	4.18±.21
Totals and means	**930**	**22.83±.06**	**2.78±.04**	**90.65±.07**	**3.21±.05**	**78.75±.09**	**4.14±.06**

We shall content ourselves, therefore, with a summary of the data. Tables 8 and 9 show, for each sex separately, the mean values and variability as regards the size characters studied in the case of each color class. Table 10 shows the influence of each mutant gene on the size characters studied. In the case of short-ear and dilution, it is possible to estimate only their

TABLE 9

Comparative variability of males of the several color classes produced by a backcross of F_1 females to the se race

		WEIGHT		BODY LENGTH		TAIL LENGTH	
	NO.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
Black	126	28.11±.19	3.20±.13	94.32±.20	3.24±.14	80.72±.28	4.50±.20
Blue se	117	27.81±.19	3.04±.13	94.36±.17	2.64±.12	80.75±.29	4.40±.20
Brown	101	30.31±.25	3.81±.18	96.41±.24	3.61±.17	82.74±.35	5.11±.24
D br se	117	28.46±.21	3.47±.15	95.33±.22	3.60±.16	81.06±.29	4.52±.20
PE Black	100	28.42±.22	3.24±.15	94.51±.23	3.40±.16	80.49±.33	4.70±.23
PE Blue se	121	26.69±.18	2.98±.13	93.12±.19	3.09±.13	79.85±.29	4.65±.21
PE Brown	136	29.46±.18	3.20±.13	95.83±.19	3.39±.13	81.53±.29	4.84±.20
PE d br se	99	27.02±.25	3.68±.18	94.42±.19	2.79±.13	81.16±.25	3.53±.18
Totals and means	**917**	**28.38±.08**	**3.60±.06**	**94.80±.07**	**3.37±.05**	**81.01±.10**	**4.60±.07**

combined effect. In other backcrosses we have found that dilution regularly increases body size, but it is clear from this cross that short-ear decreases size more than dilution increases it, so that their combined action is a decrease even greater than the increase made by brown, which, up to this time, had been found more influential than any other single gene mutation on body size. The decrease in weight amounts to 3.67 percent for females, 5.74 percent for males. How great the decreasing effect of short-ear on body size would be, apart from dilution, it is impossible at present to state. Among other gene mutations which decrease body size must

undoubtedly be included dwarf and waltzing, but as yet we have no data on their quantitative influence.

Brown, as in other backcrosses, has a tendency to increase all size characters studied. Pink-eye, on the other hand, though also derived from the larger parent race in the original cross, has a tendency to decrease size in the backcross. This tendency is manifested in all characters studied except weight in the case of females. Here some extremely fat individuals made the average weight for all pink-eyed females exceed that of the dark-eyed,

TABLE 10

Influence of particular genes on body size in the backcross to the se race, as indicated by percentage increase or decrease (−) of the average.

	FEMALES			MALES		
	WEIGHT	BODY	TAIL	WEIGHT	BODY	TAIL
Brown	.68	.60	.10	3.80	1.53	1.45
SE+Dilution	−3.67	−.40	−.74	−5.74	−1.01	−.82
Pink-eye	.74	−.09	−.76	−2.77	− .19	−.69

but as regards body-length and tail-length, pink-eyed females as well as males, fell below the dark-eyed. The effect of pink-eye in decreasing body size is small but consistent in both sexes (except for weight in females) and so may be accepted as genuine.

We are now in a position to consider the theoretical question, is it linkage with size genes borne in the same chromosomes, or is it the physiological action on growth of the mutant genes themselves which is responsible for the effects noted in these mouse crosses? The larger parent introduced mutant genes which mark four of the twenty chromosomes of the mouse, namely, (1) agouti, (2) brown, (3) dilution and short-ear, and (4) pink-eye. On the linkage hypothesis we should expect to find larger body size associated with each of these genes in the backcross segregates. From the results of our study it appears that there are no linked size genes in the agouti chromosome or the pink-eye chromosome, since agouti individuals do not consistently differ from non-agoutis, and pink-eyed segregates are actually smaller than dark-eyed, contrary to expectation based on the linkage hypothesis. Dilution is associated with larger size when short-ear is not present, and brown is regularly found in the larger-bodied segregates. For the linkage hypothesis we have then, thus far, two positive and two negative tests. But under further examination of the positive cases, the linkage hypothesis breaks down. The case of dilution and short-ear affords a crucial test. Dilution by itself increases size, but when short-ear is present, size is decreased. But dilution and short-ear are closely linked, crossovers occurring less than once in a thousand times. If linkage with a size gene were

responsible for the larger size of dilute animals, this gene should become effective irrespective of the presence of short-ear, since it would become homozygous when *either* dilution or short-ear became homozygous; but we find that dilution increases body size even when short-ear is present as a heterozygote; yet when short-ear is homozygous, size is decreased even in the presence of dilution. We are thus forced to assume a physiological action of short-ear on growth. If we concede the existence of such an effect to short-ear, there is no reason why we should not concede also to dilution an influence of a contrary character. Indeed that seems to be the only logical explanation of the observed facts. But if short-ear and dilution affect growth by their physiological action and thus influence body size, there is every reason to suppose that similar action is exercised by brown. FELDMAN (1935) has presented evidence supporting this view.

Size inheritance has long been supposed to result from the joint action of many genes, but in mammals we have hitherto lacked evidence of what these genes were or how they acted. For the mouse we are now able definitely to identify as genetic modifiers of body size the mutant genes brown, dilution, and short-ear. They act through their influence on general body size as manifested in length of trunk and tail and total body weight. This means probably that their action begins early and continues throughout growth. For dilution we have the interesting observation that besides its general action on body size, it exercises a special influence toward increase in the length of the tail, not great enough, however, to counteract the contrary influence of short-ear when present. WRIGHT, DAVENPORT, SUMNER, and others have found evidence of the existence in mammals of special genetic influences local in their action. This demonstrated action of dilution supports that interpretation. It must mean, in physiological terms, that though the dilution gene is probably active throughout growth and thus influences size of the animal as a whole, it is especially active at that stage in development when the tail is being formed.

There is a genetic difference between *Mus bactrianus* and the Japanese waltzer in length of tail. The tail in *M. bactrianus* is relatively shorter. This difference is manifested in the backcross populations. For animals of the same body size, those descended from a Japanese waltzer have longer tails than those descended from *M. bactrianus*. The backcross populations produced by d br males, when mated with F_1 females from Cross 1 and from Cross 2, may be compared as to their size indices in table 11. The animals derived from Cross 2 are seen to be about 1 percent longer-bodied and 10 percent heavier, but their tails are 3 or 4 percent shorter. This is independent of the action of dilution in lengthening the tail, because its influence is present equally in both populations, and its action is positive in both cases, as already stated. But the genetic basis on which dilution

may act is different in the two backcrosses. *M. bactrianus* has a genetic complex for shorter tail length than the Japanese waltzing mouse.

TABLE 11

Mean size of backcross animals from Cross 1 and Cross 2 compared

	WEIGHT	BODY LENGTH	TAIL LENGTH
Females, from Cross 1	22.25	92.17	77.20
Females, from Cross 2	24.85	93.38	75.30
Ratio	111.6	101.3	97.5
Males from Cross 1	28.99	96.71	80.84
Males from Cross 2	31.53	97.83	77.78
Ratio	108.7	101.1	96.2

Correlation is fairly high between body length and weight; $.684 \pm .012$ in the case of males, $.642 \pm .013$ in the case of females in the backcross to the *se* race, our largest population. Between body length and tail length the correlation is less close, owing to the greater variability of tail length probably through environmental influences. The coefficients obtained for the same backcross populations as those already mentioned were for males $.466 \pm .018$, and for females $.406 \pm .019$. It is again noteworthy that higher coefficients are obtained for males than for females, indicating that growth processes are more advanced in the case of males than of females, and to a greater extent genetically determined, accidents of development and errors of observation being relatively smaller.

SUMMARY

1. Crosses were made between females of an inbred race of *Mus musculus* having pink eyes, short ears, and dilute brown non-agouti coat, mated (1) with black-and-white Japanese waltzing mice or (2) with *Mus bactrianus*.

2. Cross 1 produced dark-eyed, long-eared, intense black F_1 individuals. Cross 2 produced dark-eyed, long-eared, intense agouti F_1 individuals. In size the F_1 animals from both crosses were nearly as large as the larger parent race, and of remarkable vigor, fecundity, and longevity.

3. F_1 individuals from Cross 1 were reciprocally backcrossed to LITTLE's dilute brown race of *Mus musculus*. In both backcrosses brown individuals were of larger body size than black ones, and dilute individuals were larger than intense ones by three different criteria, namely, (a) mature body weight, (b) body length, and (c) tail length.

4. The influence of brown was found to be greater than that of dilution as judged by body weight and body length, but as regards tail length dilution was found to be more influential than brown.

5. Of the reciprocal backcrosses, that in which the pure *musculus* (d br)

race was the mother produced consistently larger offspring as regards weight, body length, and usually as regards tail length also.

6. F_1 individuals from Cross 2 were also reciprocally backcrossed to LITTLE's dilute brown race. In these backcrosses, as in those from Cross 1, brown individuals were larger than black ones, and dilute individuals were larger than intense ones by all three criteria, weight, body length, and tail length.

7. As in the backcrosses from Cross 1, so also in the backcrosses from Cross 2, brown was more influential than dilution in increasing weight and body length, but dilution was more influential than brown in increasing tail length. This leads us to the tentative conclusion that dilution has a special effect on tail length over and above its effect on general body size.

8. Again as in the backcrosses from Cross 1, so also in the backcrosses from Cross 2, the pure *musculus* race as mother produces larger-bodied offspring than the reciprocal backcross, regularly in weight and body length, and usually in tail length also. The larger racial or individual size of the mother is thus shown to be a factor in producing large body size in her offspring. Such influence, if genetic, must be extra-chromosomal, as has been shown by LITTLE as regards the inheritance of susceptibility to spontaneous cancer in reciprocal crosses and backcrosses in mice.

9. The F_1 animals from Cross 1 were also backcrossed to the maternal parent race, the pink-eyed short-eared dilute brown race of GATES. There resulted 8 color classes, segregation occurring simultaneously for three independent characters, (1) pink-eye, (2) brown, and (3) the closely linked characters, short-ear and dilution.

10. In this backcross, as in those already described, brown individuals were larger-bodied than black ones. Pink-eyed individuals were slightly smaller than dark-eyed ones, and agouti individuals showed no consistent difference, being larger-bodied in the case of females but smaller in the case of males. Dilute individuals we should have expected to be larger-bodied than intense in this as in other backcrosses, but because of its regular association with short-ear, which strongly reduces body size, the short-eared dilute classes were actually smaller-bodied than the intense long-eared classes by all three criteria employed.

11. Linkage with size genes is an inadequate explanation of the size differences found in backcross populations. Direct physiological action on growth of the mutant genes studied is the interpretation preferred by us. As regards their influence on size, brown and dilution have accelerating effects, pink-eye has a slightly retarding, and short-ear a strongly retarding effect. Agouti probably has no effect.

12. Fairly high correlation coefficients indicate that general growth

processes rather than local ones are chiefly affected by these mutant genes, but a special local action of dilution on tail length is indicated.

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CHROMOSOME COILING IN RELATION TO MEIOSIS AND CROSSING OVER

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DURING the past five years a considerable amount of evidence has been obtained which strongly supports JANSSENS' partial chiasmatypy theory of chiasma formation. This evidence includes (1) the meiotic configurations found in trivalents and quadrivalents (DARLINGTON 1930); (2) the frequency of figure-8 chromosomes in segmental interchange rings of Pisum (SANSOME 1933); (3) the relation between the failure of chiasma formation and absence of crossing over (DOBZHANSKY 1932), BEADLE 1933); (4) the relation between chiasma frequency and crossover frequency (BEADLE 1932, DARLINGTON 1934); (5) the similarity in effect of environment on chiasma frequency and crossover frequency (WHITE 1934); (6) the behavior of heteromorphic homologues (HUSKINS and SPIER 1934); (7) the evidence for interference in both chiasma formation and crossing over (HALDANE 1931); (8) the negative correlation of frequencies for both chiasmata and crossing over in bivalents of the same cell (MATHER and LAMM 1935); (9) the types of chromatid associations at meiosis (HEARNE and HUSKINS 1935); and (10) the types of interlocking between non-homologous bivalents at meiosis (HUSKINS and SMITH 1935, MATHER 1935). Some of this evidence can not be considered as critical, because its interpretation is based on unproved assumptions; but the cumulative value of the various lines of evidence does prove the validity of Janssens' theory. The direct relation between chiasma formation and crossing over certainly facilitates cytogenetic studies and the analysis of the causal factors in crossing over, a feature not found in the hypothesis suggested by the author some years ago (SAX 1930).

Recent cytological studies of chromosome structure and behavior in mitosis and meiosis have indicated the factors which are responsible for the fundamental differences in the two types of division. Although the time of chromosome duplication is a disputed question, the general features of chromosome behavior seem to be fairly well established.

The chromonemata are in the form of *minor spirals* at anaphase in mitosis. During the formation of the daughter nucleus the minor spirals begin to uncoil, but further uncoiling is inhibited as the chromosomes pass into the resting stage. At early prophase the coiled chromosomes begin to elongate and uncoil, but at the same time the two chromatids in each chromosome begin to coil independently. By the time these *relic coils* are

largely eliminated, the new spirals are well established and continue to effect chromosome shortening until metaphase. The two chromatids of each chromosome are associated in the relic spiral, and, as the relic spiral is uncoiled, the two chromatids are twisted about each other (*relational coiling*, see fig. B). The amount of twisting is reduced as the chromatids contract, and at metaphase few twists remain in the chromosomes of most species. The chromonemata of somatic chromosomes are always coiled. Before the relic coils of the previous division are straightened out, the new spirals are well established in each chromatid. There is a tendency for homologous chromosomes to pair in certain species, but intimate association is inhibited by their coiled structure during the entire mitotic cycle.

The resting stage preceding meiosis shows the same type of chromosome structure. The tempo of the early prophase stage is slower than it is in mitosis, and the relic spirals are well straightened out before the daughter chromatids become differentiated and begin to form new minor coils. At this time the affinity between homologous chromosomes permits intimate pairing. The attraction is effective only when the chromosomes are relatively free from coils and before the chromatids of each homologue become differentiated and begin to coil independently. The chiasmata are formed, and the homologous chromosomes tend to repel each other. During this time the minor coils are developed, and during the later stages the *major coils* (plate 1) are superimposed on the minor coils. The reduction in chromosome length between pachytene and metaphase is about 10:1 and may be even greater in certain species. This contraction may be effected by a linear contraction, or perhaps a "minimum" spiral, and by minor and major coiling. Major coils are not an essential feature of meiosis (O'MARA, unpublished) and apparently do not occur in all genera. The major coils do not prevent free separation of chromatids at the first meiotic division. The interphase is so short that the minor spirals, and in some species even the major spirals, persist in the second meiotic division.

The comparison of mitosis and meiosis seems to show the basis for chromosome pairing at meiosis, but a more detailed analysis is necessary for determining the mechanism involved in crossing over. DARLINGTON (1935) has prepared a most logical theory to explain the mechanism of crossing over. The essential features of this theory are based on the following assumptions. The direction of coiling of minor spirals is the same for homologous arms of somatic chromosomes; the direction of coiling is reversed at the spindle fiber attachment point, so that the two arms of a single chromosome always coil in opposite directions; the direction of coiling does not change between the fiber attachment and the distal end of the chromosome in either somatic or meiotic chromosomes; the minor and major spirals in a given chromosome coil in the same direction; and homologous chromo-

somes pair while still coiled in relic spirals. After the pairing of coiled homologues at meiosis, the chromosomes elongate and produce a "relational" coiling at pachytene. Each chromosome now divides so that relational coiling exists in the chromatids of each homologue. The relational coiling of chromatids is in the same direction in each homologue, and the relational coiling of chromosomes is in the reverse direction (fig. A). The torsional strain induces chromatid breaks which reunite in such a way that crossing over is effected between two of the four chromatids at any one locus. "The torsion which determines relational coiling is removed by crossing over and consequently must determine it."

FIGURE A. Relational coiling of homologous chromosomes at pachytene, according to DARLINGTON'S interpretation. The chromatids of each homologue are twisted about each other in the same direction, while the chromosomes twist about each other in the opposite direction.

The theory is logical, but unfortunately most of the underlying assumptions are erroneous. If hypotheses are of more importance than facts (DARLINGTON 1935c), further analysis should be unnecessary; but perhaps a few cytological observations and an analysis of chromosome behavior will be of some value in correlating cytological and genetic behavior.

MINOR COILS AND RELATIONAL COILING IN SOMATIC CHROMOSOMES

The number of coils in somatic chromosomes can be observed only in species with relatively large chromosomes, and even in such species the direction of coiling can not be determined accurately. There does seem to be a tendency for the coiling to be in the same direction from the spindle fiber to the end of the chromosome. The number of minor spirals in a somatic chromosome is about 20–25 in Tradescantia (SAX and SAX 1935) and is about 80 in Fritillaria (DARLINGTON 1935).

Since the direction of coiling in somatic chromosomes can not be determined directly, we must rely upon indirect evidence. This evidence is based on the assumption that the relational coiling of chromatids is determined by the minor coils, either as a result of uncoiling the relic coils (DARLINGTON 1935) or by the formation of new coils in each chromatid at prophase (HUSTED, unpublished).

Relational coiling in the sister chromatids of somatic chromosomes has been studied in Trillium and Vicia. Root tips were fixed in a mixture of absolute alcohol and acetic acid, and after fixing for 15–20 hours, the root tips were smeared in aceto-carmine. In favorable preparations the rela-

tional coiling could be observed and the direction of this coiling determined for all chromosomes (figs. B and C).

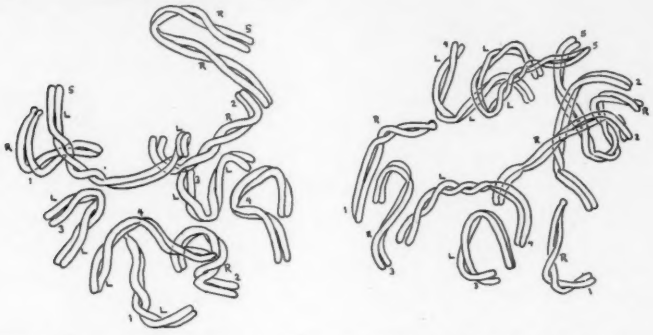

FIGURE B. Somatic chromosomes of *Trillium grandiflorum* showing relational coiling of chromatids. The five pairs of homologous chromosomes are numbered, and the direction of relational coiling is indicated. ×800.

Trillium grandiflorum has five pairs of chromosomes: (1) with a nearly terminal fiber attachment, (2) fiber subterminal, (3) sub-median fiber and short arms, (4) sub-median fiber and medium arms and (5) sub-median fiber and long arms (figs. B and C). The corresponding arms of homologous chromosomes could be determined accurately for chromosomes I, II, and V, and with a fair degree of accuracy for chromosomes III and IV. The direction of twisting of the chromatids is easily determined at late prophase or early metaphase.

The direction of relational coiling was obtained for all the chromosomes in each of twenty-five cells. The direction of twisting of chromatids in corresponding arms of homologous chromosomes is shown in table 1.

The total number of chromosome arms with right-handed or clockwise spirals is 113, and with left-handed or counter clockwise twists, 118, while 219 arms showed no twisting of chromatids at the stage of development examined. The homologous chromosomes show an approximately random distribution for direction of relational coiling. Where both of the terminal chromosomes showed relational coiling, the direction of twisting was the same in the two homologues in 9 cells and reversed in 9 cells. The total for all homologous arms was 16 with right-handed twists in both arms, 23 with left-handed twists in both arms, 36 with a right-handed twist in one homologue and a left-handed twist in the other homologue, and 150 pairs of arms which showed relational coiling in only one or in neither arm.

In any single chromosome the direction of relational coiling may be the same in both arms or may be reversed at the spindle fiber attachment point. In the chromosomes which showed relational coiling in both arms,

TABLE 1

Direction of twisting (relational coiling) in sister chromatids for homologous arms of all chromosomes in 25 somatic cells of Trillium. RR=relational coiling right in both homologues; RL or LR =right-handed twists in one homologue and left in the other; LL=left-handed twists in both homologues; O=no twist.

CHROMOSOME		DIRECTION OF TWIST						
		RR	RL OR LR	LL	RO	LO	OO	n
1.	long arm	4	9	5	3	2	2	25
2.	short arm			1	2	1	21	25
	long arm	4	3	2	8	6	2	25
3.	short arm	1		1	1	6	16	25
	long arm	1	5	4	7	2	6	25
4.	short arm		1	2	7	8	7	25
	long arm	3	4	2	5	5	6	25
5.	short arm	2	7	2	9	1	4	25
	long arm	1	7	4	3	5	5	25
		16	36	23	45	36	69	225

the direction was right in both arms of 9 chromosomes, left in both arms of 13 chromosomes, and right in one arm and left in the other arm of 24

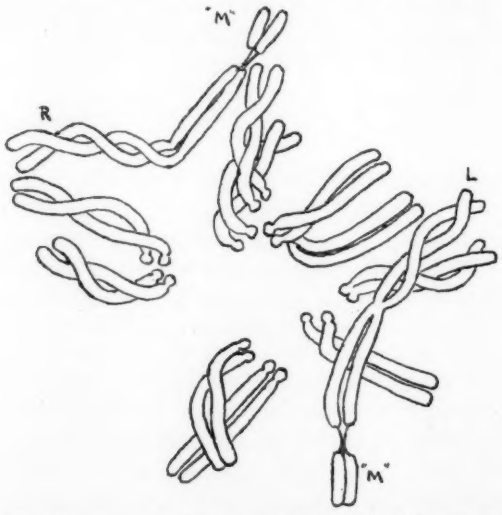

FIGURE C. Somatic chromosomes of *Vicia faba*. The direction of coiling is indicated in the long "M" chromosomes. The short chromosomes are so similar that homologues can not be identified, although the loose pairing may indicate homology. ×2000.

chromosomes. In the chromosomes with the same direction of twisting in both arms, the average number of half twists was 1.7 for the long arms and

1.3 for the short arms, while in the chromosomes with reversal in direction of twisting in the two arms, the average number of half twists was 1.5 for the long arms and 1.3 for the short arms. These observations indicate that the direction of twisting is approximately at random for either arm of a chromosome and that there is no redistribution of coiling. If coiling is transferred from one arm to the other, there should be considerable reversal of coiling within a single arm, but such reversals are very rare and were observed in only two cases.

There are six pairs of chromosomes in *Vicia faba*, five pairs with nearly terminal fiber attachments and one pair with sub-median fiber attachment points. It is not possible to determine which of the ten short chromosomes are homologues, but the pair of long "M" chromosomes is easily identified, and the two arms can be distinguished easily because the shorter arm has a very clear secondary constriction. We have analyzed the direction of relational coiling in each of the two "M" chromosomes in sixty root tip cells. The results are shown in table 2.

TABLE 2

Direction of relational coiling of chromatids in homologous arms of the "M" chromosomes of Vicia faba at early somatic metaphase.

RR	LR OR RL	LL	RO	LO	OO	n
16	25	13	20	12	34	120

Direction of relational coiling in the two arms of single "M" chromosomes of Vicia

RR	RL OR LR	LL	RO	LO	OO	n
9	18	6	41	33	13	120

The direction of twisting of chromatids about each other seems to be at random for homologous arms, with 29 showing relational coiling in the same direction and 25 showing relational coiling in opposite directions. The 120 "M" chromosomes show relational coiling in 140 arms of which 77 were right and 63 left. In single chromosomes the direction of relational coiling was the same in both arms of 15 chromosomes and reversed in 18. No case was observed where the direction of relational coiling was reversed in the long arm, but in the short arm the direction was reversed on either side of the secondary constriction in several chromosomes. The average number of half twists was found to be the same in the chromosomes with the same direction of relational coiling in both arms and those which had reversed coiling: 1.7 for the long arms and 1.1 for the short arms.

The direction of relational coiling in a single chromosome is not constant during successive cell generations. In root tip cells from a single plant the direction of relational coiling was right in both homologues of six cells,

left in both homologues of six cells, and in reverse directions in the homologues of thirteen cells.

THE MAJOR COILS IN THE MEIOTIC CHROMOSOMES OF VICIA

The direction of coiling of major spirals has been determined in a number of species, but no adequate data are available for an analysis of coiling in relation to chiasmata. The meiotic chromosomes of *Vicia faba* have been used because the chiasma frequency is high, and in favorable preparations the direction of the major coils can be determined at all loci in some of the chromosomes. We have made and examined nearly a thousand preparations, but the technical difficulties have made it impossible to get very many data on major coils in this genus.

There are six pairs of chromosomes in *Vicia faba*, one long pair with submedian fiber constrictions, and five shorter pairs with subterminal fiber constrictions. The average chiasma frequency in the material examined was 6.2 for the long "M" chromosomes and 3.0 for each of the five short chromosomes. There is a strong tendency for the chiasmata of the short chromosomes to be localized either near the spindle fiber or near the distal end, and the average number of clearly interstitial chiasmata is only 1.3 for these chromosomes.

The two chromatids of each chromosome are coiled together in a single spiral at meiotic metaphase. There are about 15 major spirals in each of the long chromosomes and about 5 or 6 major spirals in each of the short chromosomes (plate 1). The direction of coiling of a single spiral is seldom reversed between chiasmata, although reversals do occur in the loci containing the fiber attachment in the "M" chromosomes (figs. 2 and 4) and in some internodes of the short chromosomes (figs. 10 and 11). The direction of coiling seems to be at random for corresponding segments of homologous

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DESCRIPTION OF PLATE 1

Camera lucida drawings of meiotic chromosomes of *Vicia faba*, showing major coils. From permanent smear preparations. ×4000.

Figures 1–4. Bivalents with median fiber attachments. Major coiling is at random for homologues segments and for loci on either side of a chiasma. The direction of coiling may reverse at the fiber attachment.

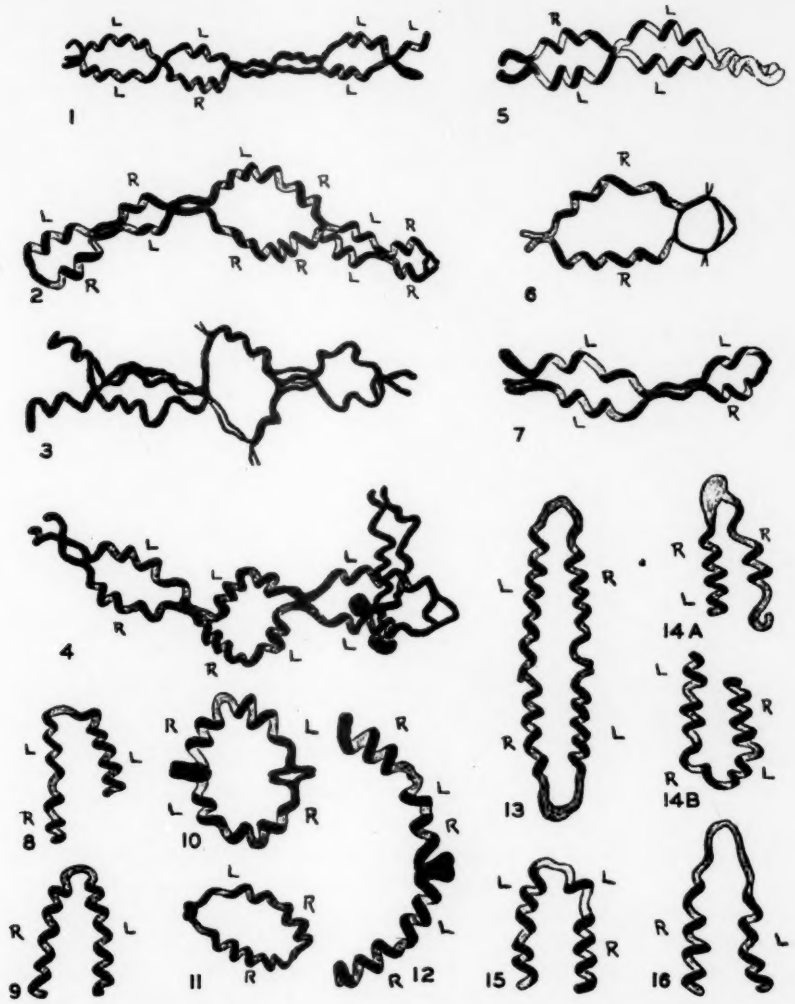
Figures 5–7. Bivalents with sub-terminal fiber attachments.

Figures 8, 9. Anaphase chromosomes.

Figures 10, 11. Two "m" bivalents with reversal of coiling between chiasmata.

Figure 12. Separation of homologues previously associated by two interstitial compensating chiasmata.

Figures 14–17. Anaphase chromosomes showing reversal in coiling in associated chromatids. Opposite directions of coiling in associated chromatids is usually caused by crossingover. The coiling relationship shown in Figure 13 is attributed to a crossover near the terminal spindle fiber rather than equational division.


1
2
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14A
14B
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16

chromosomes and for loci on either side of a chiasma. The theoretical and actual frequencies are shown in table 3.

TABLE 3

Vicia faba

Direction of coiling on either side of chiasmata; data from both M and m chromosomes.

	THEORETICAL	ACTUAL
LL×LL or RR×RR	2	2
LL×RR or RR×LL	2	1
LR×LR or RL×RL	2	3
LR×RL or RL×LR	2	2
RL×RR or LR×LL etc.	8	8
	16	16

As the chromosomes separate at anaphase, the two chromatids of each chromosome arm separate so that the direction of the major spirals can be determined for each chromatid. We have been able to analyze direction of coiling at anaphase only in the short chromosomes with the nearly terminal fiber attachments (figs. 8–16). The sister chromatids are coiled together in the same direction at metaphase, and if no chiasmata were formed, the two chromatids of an anaphase chromosome should be coiled in the same direction at all loci, with only an occasional double reversal. With random direction of coiling at metaphase, the reversals in coiling of all anaphase chromatids should be twice the chiasma frequency. In 27 anaphase chromosomes the direction of coiling of the two chromatids was the same at all loci in only 5, and the total number of reversals was 32. The reversals in coiling are not so frequent as expected with a chiasma frequency of 3.0. Part of the discrepancy may be attributed to terminal chiasmata. The average number of interstitial chiasmata is 1.3, and the calculated number based on reversal in coiling is 1.2.

Frequently the two chromatids of an anaphase chromosome coil in different directions at all loci (figs. 9,13, 16). Such configurations are the result of separating a bivalent which has a chiasma very near the spindle fiber and in which the homologues were coiled in opposite directions.

In some cases the two homologues can separate without untangling their chromatids. Figure 12 represents the separation of chromosomes previously associated by two compensating interstitial chiasmata. The association of the very short arms of the "m" chromosomes seems to be effected without chiasma formation (fig. 6). The fiber attachment points are occasionally stretched out from the chromonema, and in these cases they appear to be double (figs. 3 and 6).

One case was found where interlocking of non-homologous chromosomes provides evidence in support of the JANSSEN theory of chiasma formation

(fig. 4). The short chromosome is locked in the "M" bivalent distal to the first chiasma from the fiber attachment.

INTERLOCKING OF CHROMATIDS

Whenever two or more chiasmata are formed on one side of the fiber attachment, there should be some interlocking of chromatids at metaphase and early anaphase. In *Vicia faba* there are usually two chiasmata distal to the fiber in the "m" chromosomes, and about three in each arm of the "M" chromosomes. In the "M" chromosomes chromatid interlocking

FIGURE D. Bivalent chromosomes of *Vicia faba* at first meiotic anaphase, showing chromatid locking in a, d, and e. A symmetrical (free) bivalent is shown in c.

could occur in either arm, but only in the single arms of the "m" chromosomes. In 30 such arms chromatid interlocking was found in 5 cases, or about 17 percent. Chromatid interlocking is shown in figure D (a, d, and e) while a symmetrical "M" bivalent is shown in c. The proportions of these various configurations are of value in an analysis of the mechanism of crossing over.

CHROMOSOME STRUCTURE AND BEHAVIOR IN RELATION TO CROSSING OVER

The analysis of relational coiling of chromatids in somatic chromosomes of Vicia and Trillium provides indirect evidence that the direction of the minor coils is at random for homologous chromosomes, that the direction of minor coiling in the two arms of a single chromosome is at random, and that the direction of coiling is usually the same in any one arm of a somatic chromosome.

The direction of coiling of the major spirals at meiosis has been analyzed in several genera. The paired chromosome arms may coil in the same direction or in opposite directions, apparently at random, in Tradescantia (NEBEL 1932, SAX and HUMPHREY 1934); Sagittaria (SHINKE 1934);

Rhoeo (SAX 1935); Trillium (MATSUURA 1935); Lilium (IWATA 1935); and in Vicia. The direction of coiling may change at chiasmata and occasionally at other loci (HUSKINS and SMITH 1935, MATSUURA 1935) and appears to change at random at chiasmata in Vicia. The major coils may reverse their direction of coiling at the spindle attachment, but there is a strong tendency for the direction of coiling to be the same in both arms of a meiotic chromosome in Tradescantia and Rhoeo. DARLINGTON (1935) assumes that major coiling is in the same direction in homologous chromosomes of Fritillaria, and that the two arms of a single chromosome must coil in opposite directions; but he presents no data or references to support these assumptions, and in the few figures of meiotic chromosomes showing major coils, he fails to indicate the direction of coiling where it is not in accord with his hypothesis (1935b, fig. 1).

The random coiling in the two arms of a somatic chromosome proves that minor coiling is not caused by the rotation of the spindle fiber attachment point. In fact the normal contraction of X-ray induced fragments with no fiber attachments shows that the spindle fiber has no causal effect on chromosome contraction (HUSKINS and HUNTER 1935, RILEY, O'MARA, HUSTED, unpublished). The relational coiling in homologous chromosomes is at random in mitosis. The direction of major spirals is at random, or nearly so, in homologous chromosomes. The direction of coiling of major spirals is not dependent on the direction of coiling of minor spirals, because the minor spirals usually coil in the same direction in any one chromosome arm, while the major spirals may change their direction at a chiasma in the paired arms of homologous chromosomes.

All these observations are contrary to DARLINGTON'S assumptions, and most of them are not in accord with his recent hypothesis on the mechanism of crossing over. There is additional evidence which seems to invalidate this hypothesis.

Several hypotheses have been presented to explain the mechanism of crossing over demanded by JANSSENS' chiasmatypy theory. The first was suggested by MORGAN in 1919, and is based on the assumption that the two homologues are twisted in one direction. Breaks occur in two of the four chromatids, and these reunite in new associations. A modification of this theory was suggested by WILSON and MORGAN in 1920. The second hypothesis was proposed by BELLING (1933). He assumed that the chromosomes are single at the time of pairing; that they form half twists or overlaps at early pachytene, and when the chromomeres of each chromosome split, the connecting fibers may be formed between chromomeres of homologous chromosomes. The third theory is DARLINGTON'S relational coiling hypothesis, which demands that the relational coiling of chromatids causes relational coiling of chromosomes in a reverse direction, and that the

torsional strain induced causes chromatid breaks with reunions in new associations.

There is little direct cytological evidence to support any one of these hypotheses, but we can test the different theories by comparing the theoretical chromatid relationships which must be produced with the configurations actually observed.

The only adequate data are those on the chromatid relationships of meiotic chromosomes of Melanoplus (HEARNE and HUSKINS 1935). In the chromosomes with two chiasmata each, there are four types of chromatid relationships: free, chromosomes locked, chromatids locked, and continuous. These types are illustrated by diagrams in figure E. As the meiotic chromosomes begin to separate at anaphase, it is difficult to distinguish the various types of configurations with the exception of chromatid interlocking. In 59 bivalents of Melanoplus there were 24 (40 percent) free, 7 (12 percent) with chromosomes locked, 10 (17 percent) with chromatids locked, and 18 (30 percent)continuous. In early anaphase chromosomes of Vicia 17 percent showed chromatid locking, and in Lilium (MATHER 1935) there was less than 17 percent of chromatid locking.

Where there are two chiasmata on the same side of the fiber attachment, we should expect to obtain the four types of bivalents shown in figure 5. Configurations more complicated than simple chromatid locking are not found in Melanoplus and are rarely found even in species with higher chiasma frequencies. Any valid theory of crossing over should give the four types of bivalents observed.

Let us first examine the theory based on the suggestions of WILSON and MORGAN. The four chromatids lie parallel and twist together in one direction. The torsion causes a break in two non-sister chromatids and a subsequent reunion in a new association. If the torsion is in the same direction at all loci, the greater stress may lie on either side of the chromatid breaks, so that, in effect, the rotation of broken chromatids will be at random. The essential features of torsional strain, the chromatid breaks, types of crossover chromatids and chromatid relationships in the bivalents are shown in figure E and table 4. The application of this hypothesis produces 12.5 percent of free bivalents, 12.5 percent of chromosome locking, 25 percent of chromatid locking, and 50 percent of continuous association of chromatids.

We have attempted to analyze BELLING'S and DARLINGTON'S hypotheses in the same way. In the case of BELLING'S theory, we have assumed that the homologous chromosomes can be twisted about each other at random, or in only one direction. In applying DARLINGTON'S theory we have assumed that the amount of relational coiling in chromatids is the same as the relational coiling of chromosomes, but in the reverse direction (fig.

TABLE 4

Crossovers and types of bivalents resulting from two successive crossovers. Torsion of four parallel chromatids. See diagram in figure E.

TORSION	CHROMATIC BREAKS		CROSSOVER CHROMATIDS	TYPE OF BIVALENT
1–2	A	A	2 non-crossover	Chromatid lock
	B	B	2 compensating	
1–3	A	A	2 non-crossover	Free
	B	B	2 compensating	
1–2	A	A′		
	B	B′	4 non-compensating	Chromatid lock
1–3	A	A′		
	B	B′	4 non-compensating	Chromosome lock
1–2	A	A		
	B	B′	4 compensating	Continuous
1–2	A	A′		
	B	B	8 non-compensating	Continuous
1–3	A	A		
	B	B′	4 non-crossover	Continuous
1–3	A	A′		
	B	B		Continuous

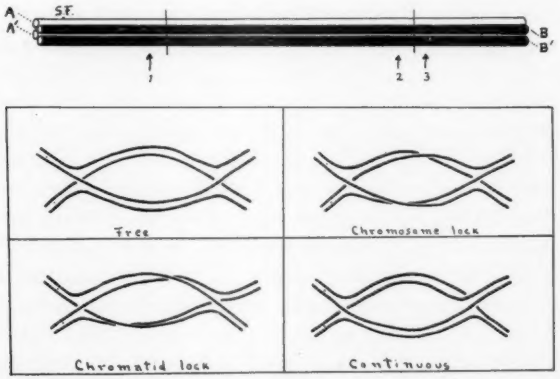

FIGURE E. Pachytene association of chromatids before crossing over, with torsional strains and points of crossing-over indicated. Below are the four types of bivalents produced by the torsion, and the only types observed cytologically in Melanoplus.

A). The configurations produced in these various theories are shown in table 5.

The rotation-torsion hypothesis gives all four types of bivalents observed cytologically, and only these types. If the torsional stress is not at random, the proportions of free, chromosome locked, and chromatid locked bivalents will be changed. But even with the maximum production of free bivalents, the frequency would not equal the percentage observed in

TABLE 5

The configurations produced by different theories of crossing over in chromosomes with two chiasmata distal to the fiber.

HYPOTHESIS	TYPES OF BIVALENTS IN PERCENT				
	FREE	CHROMOSOME LOCK	CHROMATID LOCK	CONTINUOUS	COMPLEX LOCK
WILSON-MORGAN	12½	12½	25	50	0
BELLING random	25	18¾	6¼	25	25
BELLING same	0	0	50	50	0
DARLINGTON	0	0	25	50	25
OBSERVED					
Melanoplus	40	12	17	30	0
Lilium			17		
Vicia			<17		

Melanoplus. However, the theoretical and observed frequencies of the other classes do not deviate so greatly, considering the number of observations and the difficulties in interpreting the chromatid relationships. The percentage of chromatid interlocking in Melanoplus, Lilium, and Vicia is considerably less than the theoretical expectation, but observations based on anaphase figures would fail to detect all the chromatid locking.

This torsion hypothesis will produce no chromosome locking and does produce complex interlocking if twists occur in sister-chromatids or if sister strand crossing over occurs between the two chiasmata.

If BELLING'S and DARLINGTON'S theories have been interpreted correctly, it appears that neither one is valid. If the homologous chromosomes are twisted at random at early pachytene, BELLING'S theory would be expected to produce very few chromatid locks, and 25 percent of complex interlocking. If relational coiling is in the same direction at all loci of the paired chromosome arms, as seems probable, we should expect only chromatid locking and continuous associations of chromatids. BELLING'S theory is also difficult to reconcile with the many observations that the chromosomes split at least one cell generation in advance of division.

The application of DARLINGTON'S theory gives neither of the first two classes of bivalents and does produce complex interlocking. The cytological evidence also shows that most of DARLINGTON'S "observations" regarding relational coiling are incorrect.

The torsion theory based on the suggestions of WILSON and MORGAN seems to be most nearly in accord with the cytological observations. This theory demands the following cytological interpretation. The chromosomes at meiotic prophase elongate until the relic coils of the preceding mitotic division are practically eliminated. There is little or no relational coiling

of the chromatids in each chromosome. The homologous chromosomes pair so that all four chromatids are approximately parallel, or at least tend to lie in the same relative quadrant at all loci. The formation of minor or subminor coils is now initiated, and all four chromatids coil in the same direction. The torsion induced by the initiation of the new coiling causes a relational coiling of all four chromatids. This relational coiling produces crossing over at late pachytene or at early diplotene. Much of the relational coiling of the homologues may persist during diplotene, but further contraction of the chromosomes, effected by major and minor coils, eliminates relational coiling at the later stages. The uncoiling of the homologous chromosomes will lead to no complex associations so long as there is no independent relational coiling of the chromatids of each chromosome.

This cytological interpretation of crossing over does not appear to be irreconcilable with the cytological evidence. It is difficult, however, to imagine the mechanism which would produce the precise crossing over demanded by the genetic evidence, and limit it to homologous chromatids, but this difficulty is inherent in any torsion theory of crossing over.

SUMMARY

During somatic prophase the two chromatids of each chromosome are twisted about each other. This relational coiling of chromatids has been analyzed in the somatic chromosomes of *Trillium grandiflorum* and *Vicia faba*. The direction of relational coiling is approximately at random for corresponding arms of homologous chromosomes and for the two arms of a single chromosome. In any one chromosome arm the direction of relational coiling is rarely reversed. There is no evidence that relational coiling is transferred from one arm to the other arm of the same chromosome. It is believed that relational coiling is conditioned by the nature of the minor coils in the somatic chromosomes, so that a study of relational coiling permits an indirect analysis of minor coiling.

At meiosis major spirals are superimposed on the minor coils, at least in a number of plant species. The direction of coiling of these major spirals can be observed directly. In the various species which have been studied, the direction of coiling is approximately at random for homologous chromosomes. In *Vicia faba* the direction of coiling of major spirals is at random on either side of a chiasma. Occasionally it may be reversed at other loci. The direction of coiling of major spirals is not necessarily dependent upon the direction of coiling of the minor spirals.

The various theories of crossing over, based on JANSSENS' partial chiasmatypy hypothesis, have been analyzed in relation to chromosome behavior and chromatid relationships. The torsion theory proposed by

WILSON and MORGAN in 1920 seems to be most nearly in accord with the cytological observations.

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HARELIP IN THE HOUSE MOUSE

I. EFFECTS OF THE EXTERNAL AND INTERNAL ENVIRONMENTS

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INTRODUCTION

THE character "harelip and cleft palate" is definitely inherited (REED and SNELL, 1931). Though there are various degrees of expression of harelip and cleft palate, the general condition, regardless of the types of clefts, will henceforth be designated simply as "harelip." Harelip is not inherited as a simple dominant or a simple recessive but seems to depend upon either one recessive gene with modifiers or the cooperation of a small number of cumulative genes. If the first hypothesis is correct, it must be assumed that when the recessive gene for harelip is homozygous, the mouse may show any grade of harelip from the most extensive bilateral clefts to a normal mouth without clefts, depending upon whatever genetic and environmental modifiers had an effect upon the embryo. The phenotypically normal, but genetically harelip, animals will be designated "normal overlaps."

It is usually easy to determine at birth whether a mouse is phenotypically harelip. Occasionally the cleft or clefts are so slight that they are hardly noticeable. Probably in several cases there have been slight lip and palate defects in genetically harelip animals which were not noticed. Such animals would have been erroneously classified as "normal."

Environmental influences greater than ordinary may be suspected in connection with variable characters such as harelip. These effects may be detected if found to occur in correlation with differences in litter size, weight, age of mother, and the like.

EXPRESSION OF HARELIP AFFECTED BY THE EXTERNAL ENVIRONMENT

One can not hope to measure these except with inbred stocks. Following the discovery of harelip in 1930, the stocks have been intensively inbred. At present there are three highly inbred lines and their substrains, all descended from the original harelip albino stock without outcrossing except in the early generations. For the last 10–14 generations a few matings have been made between daughter and father but nearly all have been between sister and brother.

The extent to which these three lines have been inbred and the variation in the percentages of harelip produced as inbreeding proceeded are shown

in figure 1. Only the main branches of any of the lines are shown; the less important branches are usually of short duration before becoming extinct for one reason or another. The percentages of harelip in line 1 have increased as inbreeding continued whereas in line 3 they have decreased (down

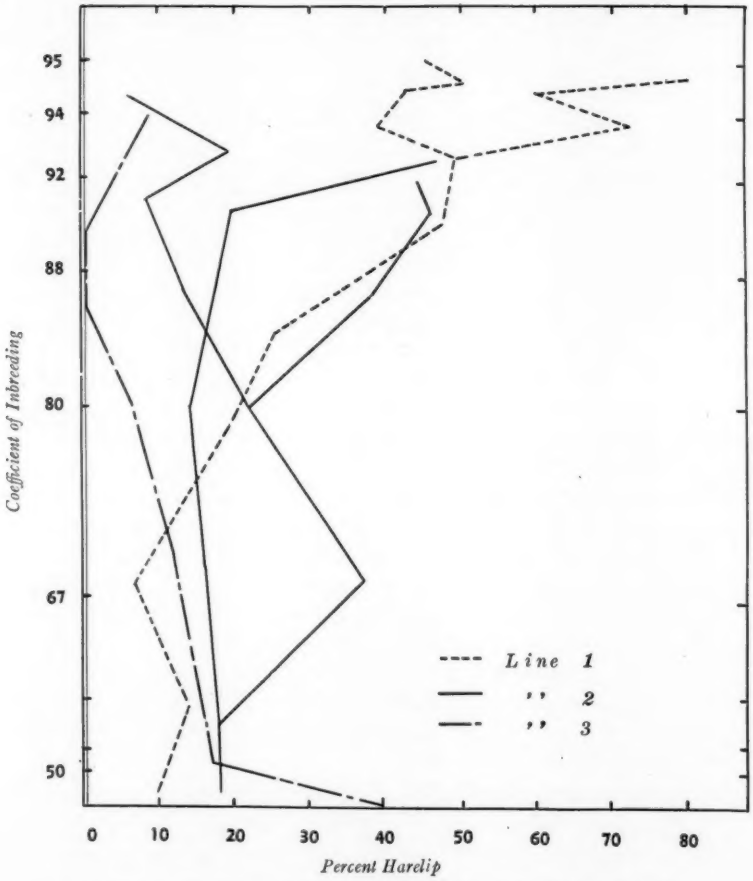

FIGURE 1. The main branches of three highly inbred harelip lines studied in this paper.

to 0 percent). Each point of each line in figure 1 was determined by the average percentage of harelip in 2–10 litters of the generation plotted. Line 1 has at this time reached a coefficient of inbreeding (WRIGHT'S) of over 95 percent; this is equivalent to some fourteen generations of brother

by sister inbreeding. We may assume that there is now practically complete homozygosity in each of these lines.

The difference in percentage of harelip between lines 1 and 3, in the last generations at least, is sufficiently great and consistent to indicate real genetic differentiation. In the following paper an attempt will be made to determine whether this difference is due to different combinations of the genes modifying one recessive essential for any harelip production or whether harelip depends upon the cumulative action of several genes of similar value, different groups of which produce characteristic frequencies of harelip.

Our first problem, however, is to test whether some of the variability within any one strain is due to environmental factors. The data for the study of environmental factors are derived from lines 1, 2, and 3 and their sublines (1a, 2a, 2b, etc.). In investigations such as that of the relation between sex and harelip expression, crossbred animals have been included with the inbred animals of lines 1, 2 and 3 but all cases where animals are included that have not been inbred for several generations are specified.

Litter size

It was shown many years ago that litter size is probably the most important single factor in determining the birth weight of guinea pigs, and that the larger the litter, the shorter the gestation period (MINOT 1891, WRIGHT 1921 and 1922, and EATON 1932). Probably the situation is similar in mice; GATES (1925) reports that in mice the birth weights tend to vary inversely with the litter size, while KING (1915) has shown that weight of rats at birth is in direct proportion to the length of the gestation period. Litter size accordingly would seem to be a factor worth investigating in its relation to harelip.

If there is a competition between embryos for some substance particularly concerned with normal development of the jaw and palate, supplied by the mother only in a certain quantity, the smaller the litter the more of the substance for each embryo. Accordingly, small litters should have a lower percentage of harelip than large ones.

Litter size was investigated in 525 litters. The first and second litters of each of the mothers are included and as many following litters in consecutive order as it was possible to include. Each of the litters was observed within 24 hours of birth. All the litters from any one mother had the same father. All the parents were from the inbred stocks (lines 1, 2 and 3) derived in 1930 from Bagg albinos.

Many precautions have been taken in selecting proper litters for the study of environmental effects, with the intention that all material might be strictly comparable. In the statistical treatment, the standard error has been used in preference to the probable error.

TABLE 1

Distribution of harelip litters by size and by number of harelip (hp=harelip animals)

GROUP	SIZE OF LITTER	OBSERVED NO. OF LITTERS WITH						TOTAL LITTERS	EXPECTED NO. OF LITTERS WITH						
		0hp	1hp	2hp	3hp	4hp	5hp		0hp	1hp	2hp	3hp	4hp	5hp	6hp
	1	3						3	2.7	0.3					
	2	10	3					13	10.4	2.5	0.1				
	3	24	8	1				33	21.1	10.1	1.7	0.1			
	4	32	6	1				39	24.9	11.8	2.1	0.2			
A	5	25	21	4				50	28.6	16.9	4.0	0.5			
q=.1059	6	33	19	4	4			60	30.7	21.8	6.4	1.0	0.1		
	7	27	20	9	2			58	26.5	22.0	7.8	1.5	0.2		
	8	15	10	6	5			36	14.7	13.8	5.8	1.5	0.2		
	9	10	4	3				17	6.2	6.7	3.2	0.9			
	10		2	7				9	3.2	3.5	1.9	0.6			
	11		1					1	.3	.4	0.3	0.0			
	12	1	2					3	.8	1.1	0.8	0.3			
Total		180	96	35	11			322	169.9	110.9	34.1	6.6	.5		
	1	2	1					3	2.1	0.9					
	2	5	3	1				9	4.6	3.7	0.7				
	3	4	2	3	1			10	3.6	4.6	1.8				
	4	8	4	3	1			16	4.1	6.7	4.1	1.1			
B	5	8	5	9	4	2		28	5.1	10.4	8.4	3.4	0.7		
q=.2889	6	6	6	6	6		1	25	3.2	7.9	8.0	4.4	1.3	0.2	
	7	4	4	13	5	3		29	2.7	7.6	9.2	6.3	2.5	0.6	0.1
	8	1	2	4	3	2	3	15	1.0	3.2	4.5	3.7	1.9	0.6	0.1
	9			2		1		3	0.2	0.5	0.9	0.8	0.5	0.1	0.0
	10		1	2	1	2		6	0.2	0.9	1.5	1.6	1.1	0.6	0.1
Total		38	28	43	21	10	4	144	26.8	46.4	39.1	21.3	8.0	2.1	0.3

TABLE 1 (*Continued*)

TABLE 1 (*Continued*)

GROUP	SIZE OF LITTER	OBSERVED NO. OF LITTERS										TOTAL LITTERS
		0hp	1hp	2hp	3hp	4hp	5hp	6hp	7hp	8hp	9hp	
	1	1	1									2
	2	1	1									2
	3	3	1	3								7
	4	3	2	3	2							10
C	5		1	1	5	2						9
q = .4859	6		1	3	6	2						12
	7				6	1	1	1				9
	8					3		1				4
	9						1	1		1		3
	10											
	11										1	1
Total		8	7	10	19	8	2	3		1	1	59
		EXPECTED NO. OF LITTERS										
	1	1.0	1.0									2.0
	2	0.5	1.0	0.5								2.0
	3	0.9	2.7	2.6	0.8							7.0
C	4	0.7	2.6	3.7	2.4	0.6						10.0
	5	0.4	1.5	2.9	2.7	1.3	0.2					9.0
q = .4859	6	0.2	1.3	3.0	3.8	2.7	1.0					12.0
	7	0.1	0.6	1.6	2.5	2.4	1.4	0.4				9.0
	8	0.0	0.2	0.5	0.9	1.1	0.8	0.4	0.1			4.0
	9		0.1	0.3	0.5	0.8	0.7	0.4	0.2	0.0		3.0
	10											
	11		0.0	0.0	0.1	0.2	0.2	0.2	0.2	0.1	0.0	1.0
Total		3.8	11.0	15.1	13.7	9.1	4.3	1.4	0.5	0.1	0.0	59.0
GRAND TOTALS A–C												
Observed		226	131	88	51	18	6	3		1	1	525
Expected		200.5	168.3	88.3	41.6	17.6	6.4	1.7	0.5	0.1	0.0	525.0

In the quantitative determinations of the amounts of variance affected by various influences such as litter size a smaller population has been used. This is composed of animals from line 1 and its recent branch 1a. This population of 1284 animals will be referred to as the "small population," and will be used for all biserial eta and tetrachoric correlations.

Most environmental factors would act on litter mates alike. Malnutrition, disease and age of mother, if effective, would tend to make litters have excesses of either harelip or normal as the conditions might determine. A method of ascertaining whether or not there are disturbances in the distribution of normal and abnormal animals among the litters has been developed by WRIGHT (1934a) and will be used here. It is possible to calculate the expected occurrence of harelip in litter mates as well as the number of harelip animals expected for each size of litter.

Each mother may be placed in one of three groups in respect to the percentage of harelip young produced in all her litters. In table 1, Group A includes all the litters from mothers which produced up to 21 percent of harelip young (as an average of the fraternity). Group B includes all the litters from mothers which produced from 21 to 40 percent of harelip young. Group C includes all the litters from mothers producing from 41 to 60 percent of harelip young.

The distribution of harelip in litters of each size, expected under random sampling, can be calculated as follows. Let q be the chance of abnormal development in the group in question, and $(1-q)$ the chance of normal development. In litters of 2 the chance that both will be normal is $(1-q)^2$; that one will be harelip and one normal is $2q(1-q)$; and finally that both will be harelip is q^2. In litters of 3 the chances of 0, 1, 2, 3, harelip are the appropriate terms in the expansion $[(1-q)a+qA]^3$ where a stands for normal and A for abnormal. The expectations for larger litters are given by expansion of the appropriate power of the binomial.

The degree of agreement of the grand totals of table 1 is shown in table 2. Note that there were many more litters observed than expected in which there were no harelip offspring and a considerable deficiency of litters containing one harelip animal.

TABLE 2

Relation of the observed number of litters containing 0–9 harelip offspring to the number expected. (hp = harelip)

	0 hp	1 hp	2 hp	3 hp	4 hp	5–9 hp	Total
Observed (m+x)	226	131	88	51	18	11	525
Expected (m)	200.5	168.3	88.3	41.6	17.6	8.7	525.0
x^2/m	3.24	8.27	0.00	2.12	0.01	0.61	14.25

There are six classes in the table and two degrees of freedom are lost—one by accepting the total number and one (approximately) by accepting

the proportion of harelip in each group. With four degrees of freedom the chance that such deviations would occur from random sampling is less than 0.01 and because of this small probability, the deviations are undoubtedly significant. The deviations are of the order expected if there were a tendency for members of a litter to be similarly affected by environmental factors. Among other factors, age of mother, if significant, could have caused deviations of this order.

Table 3a presents the data for the large population (lines 1, 2 and 3). It will be noted that small litters are deficient in harelip, litters of 5–7 agree with expectation while there is an excess of harelip in large litters. The χ^2 test shows that the correlation between large litters and higher percentage of harelip is undoubtedly significant; total $\chi^2 = 16.04$ with only two degrees of freedom.

TABLE 3a

Significance of the relation between size of litter and number of harelip in the litter (whole population)

Litter size	1–4	5–7	8–12	Total
Observed hp (19.6%) (m+x)	74	345	178	
Expected hp m	103.3	339.3	154.4	
Deviation x	−29.3	+5.7	+23.6	
x^2/m	8.30	0.96	3.62	12.88

$$\chi^2 = \left(1 + \frac{.196}{.804}\right) \Sigma \frac{x^2}{m}$$

$\chi^2 = 1.244 \times 12.88$ Total $\chi^2 = 16.04$

TABLE 3b

Significance of the relation between size of litter and number of harelip in the litter (small population)

Litter size	1–4	5–7	8–12	Total
Observed hp (25.1%) (m+x)	48	179	100	327
Expected hp m	55.3	184.6	87.1	327.0
Deviation x	−7.3	−5.6	+12.9	
x^2/m	0.96	0.17	1.91	3.04

$$\chi^2 = \left(1 + \frac{.251}{.749}\right) \Sigma \frac{x^2}{m}$$

$\chi^2 = 1.335 \times 3.04$. Total $\chi^2 = 4.06$

In the small population the correlation is of the same type as that for the large population although χ^2 is not significant. There can be no doubt, however, that the effect of size of litter is present in the small population even though not in such a pronounced degree (table 3b).

The actual correlation was calculated for the small population (1284 animals). Biserial eta squared is equal to 0.035. This means that 3.5 percent of the total variation in harelip production of the small population is due to the litter size. WRIGHT's formula for biserial eta squared is, eta squared $= \sigma^2/1+\sigma^2$. The sigma is that for the total group of arrays.

Age of the mother

KING (1917) and others have found a relationship in rats between age of mother and birth weight of young. In all cases the young from mature dams were heavier than those from young females. If the age of the mother is an important factor in the expression of harelip, young mothers might be expected to produce a higher percentage of harelip young than mature ones.

When the mean percentage of harelip produced by mothers of the various ages is calculated, we find larger fluctuations than we should expect from chance alone. The percentage of harelip young is high in the litters of 2 and 4 months old mothers but, by exception, is very low in the case of mothers 3 months old. Subjected to all manner of tests, this low percentage from 3 months old mothers remains. It is always consistent and significant. The percentage for mothers 4–7 months of age is about that found for 2 months old mothers, but for mothers 8–10 months old, the percentage is very low (table 4).

It was thought necessary to study those mothers which were still producing litters at 7–10 months of age. By selecting only those mothers which produced offspring from the time they were about 2 months of age until they were about 7–10 months old we avoid fluctuations in the data resulting from differences in harelip frequency of different fraternities of young. As an illustration, if one mother produced an average of 50 percent of harelip progeny in all her litters but produced none after she was 6 months old, while a second mother averaged only 25 percent harelip, but produced them through 10 months of age, there would be a spurious drop in harelip production after 6 months of age observed in the combined data of the two females. Mothers which are more nearly homozygous for genes influencing harelip might produce higher percentages of harelip but fail to produce young after 6 months of age. Therefore the following calculations are from mothers which produced about one litter a month from the age of 2 months to 10 months.

The lower part of table 4 shows the significant drop in harelip production at 3 months of age. This drop, which is the only statistically significant fluctuation, is impossible to explain physiologically at present. The drop in harelip percentage comes suddenly with mothers 10 weeks of age, while the abrupt rise begins at about 15 weeks. Examination of several groups of females separately shows, consistently, the drop in percentage of harelip produced by 3 months old mothers.

A χ^2 table was made to test whether there is an association between harelip expression and age of mother. In this table there were eight age groups against the two alternatives harelip and not-harelip, seven degrees of freedom, and a total χ^2 of 18.01. It is probable that such a large χ^2 would

result from chance factors alone only about once in one hundred times. Therefore there is a significant relationship between the expression of harelip and the age of the mother.

TABLE 4

The percentages of harelip young born to mothers from 2–11 months old

AGE OF MOTHER (MONTHS)	NO. OF LITTERS	HARELIP YOUNG (PERCENT)
2	45	30.9±3.5
3	83	19.6±2.2
4	85	25.2±2.4
5	55	30.9±3.4
6	38	23.5±3.5
7	23	22.0±4.1
8	17	17.2±4.3
9	10	16.1±4.7
10	3	11.3
11	2	0.0

Data from mothers still producing at 7–10 months

AGE OF MOTHER (MONTHS)	NO. LITTERS	HARELIP YOUNG (PERCENT)	DIFFERENCE
2	52	29.9±3.2	
			2.2^{σ}
3	100	21.2±2.2	
			2.6^{σ}
4–5	172	28.8±2.0	
			0.7^{σ}
6–7	95	31.5±3.1	
			1.8^{σ}
8–10	56	23.3±3.5	

As a descriptive statistic it would be interesting to know how great the correlation is between age of mother and the expression of harelip. The use of the coefficient of correlation (r) is valid only when the regression is rectilinear; therefore it is better to use the correlation ratio (eta), which is suitable for both rectilinear and curvilinear distributions. Biserial eta was used in this case where the two alternatives, harelip and not-harelip, are correlated with the different ages of mother. Biserial eta was found to be 0.164. The error of this correlation is not included, the χ^2 test having shown the relation between expression of harelip and age of mother to be significant. The symbolism and formulae employed in determining eta were taken directly from WRIGHT (1934b, p. 513–514).

We have found eta = 0.164. Eta squared is 0.027, indicating about 3 percent determination of the total variance of harelip by the age of mother. Considering the inexplicable drop in harelip production of 3 months old

mothers, this finding of 3 percent variance has not been considered in our final analysis; it is merely indicated that age of mother is a factor, but its quantitative determination is left in abeyance.

WRIGHT'S (1934b) valuable paper on polydactyly shows "that immaturity of the mother has a much greater influence on the development of an atavistic little toe by the young than on a number of characters (such as mortality at birth) in which an effect would seem more likely on *a priori* grounds." I assume that the age effect in the case of polydactyly may parallel the age effect on harelip. I assume that if there is an effect of age, it is a direct physiological one acting upon the early embryo.

Time interval between litters

The gestation and nursing periods in mice each take about three weeks. Females often carry a litter while nursing the litter recently born and may, for a period of several months, both carry and nurse successive litters at the same time, weaning one litter at the birth of the next one. Such a reproductive load could conceivably affect expression of harelip. We may investigate this possibility by use of the time interval between successive litters. If a litter is born 3 or 4 weeks after the birth of the previous litter, it is likely that this litter was carried while the previous litter was nursing. If, however, the litter is born 6 weeks after the previous one, this previous litter will have been weaned before gestation of the later one. We may compare the percentages of harelip contained in litters born 3, 4, 5, or 6 or more, weeks after the birth of the previous litter. Percentages of harelip for each litter were not calculated, but the mean percentage of harelip of all litters born at the specified period. The material is all from the small population.

NO. OF LITTERS	NO. OF WEEKS ELAPSED SINCE BIRTH OF THE PREVIOUS LITTER	PERCENT HARELIP
89	3	35.0
25	4	39.8
12	5	44.5
35	6 or more	30.6

We may well combine the data for 3 and 4 weeks (114 litters with 36.0 percent harelip) and for 5 and 6 weeks (47 litters with 34.4 percent harelip). The difference between these percentages could easily be due to chance alone. There is, then, no significant effect of the length of the time interval between litters upon the expression of harelip.

A further related attempt was made to analyze effects of the condition of the mother on the expression of harelip in the young. One might expect to find an association between the nursing of the previous litter and a

TABLE 6

Harelip (H) in relation to age of mother (A) and birth rank (B)

BIRTH RANK	AGE OF MOTHER IN MONTHS																TOTAL	
	2		3		4		5		6		7		8		9–10			
	NO. YOUNG	PERCENT HARELIP	NO. YOUNG	PERCENT HARELIP	NO. YOUNG	PERCENT HARELIP	NO. YOUNG	PERCENT HARELIP	NO. YOUNG	PERCENT HARELIP	NO. YOUNG	PERCENT HARELIP	NO. YOUNG	PERCENT HARELIP	NO. YOUNG	PERCENT HARELIP	NO. YOUNG	PERCENT HARELIP
1	81	30.9	100	17.0	36	33.3											217	24.9
2			70	21.4	101	28.7	56	21.4									227	24.7
3					66	42.5	115	27.8	64	26.6	4	25.0					249	31.3
4							41	34.2	15	25.2	63	28.6	7	28.6			262	27.5
5									47	29.8	61	16.4	48	18.8	12	33.3	168	22.0
6											16	25.0	64	17.2	22	27.3	102	20.8
7													3	0.0	44	18.2	47	17.0
8															12	16.7	12	16.7
Total	81	30.9	170	18.8	203	34.0	212	27.4	262	26.3	144	22.9	122	18.2	90	22.2	1284	25.6

higher percentage of harelip in the next litter, if this next litter were *in utero* while the previous litter was nursing. This was not the case. If the previous litter was nursed, 27 of the litters which followed had a higher percentage of harelip than that of each corresponding previous litter; the remainder of the litters that followed (38) had a lower percentage of harelip than each of the corresponding previous litters. If the previous litter was removed at birth and not nursed, 63 litters arriving within the next month contained a higher percentage of harelip than the corresponding previous one, and 62 contained a lower percentage.

Lactation has no apparent influence upon harelip expression.

Birth rank

The correlation between expression of harelip and age of mother ($\eta_{HA} = -.16$) allows us to be fairly certain that a correlation between birth rank and expression of harelip will be found because age of mother and birth rank bear an obvious relationship to each other. The correlation between birth rank and expression of harelip proved to be $\eta_{HB} = -.12$. This is not as great as the correlation between age of mother and harelip expression and indicates that age of mother and not birth rank is a factor involved in the variation of harelip expression. Table 6 presents the data concerned with both birth rank and age of mother.

The correlation between age of mother and birth rank is of course high ($r_{AB} = +.96$). It is concluded that birth rank has no effect on the expression of harelip.

Seasonal variation

It would be reasonable to assume that a character such as harelip might be influenced in its expression by seasonal changes. The temperature of the mouse room was held fairly near 70°F during the winter months, but through late spring, summer, and autumn there were considerable fluctuations due to the effect of heat from outside. In spite of such temperature and seasonal changes there seems to have been no significant variation in the percentage of harelip young (table 7).

Though there seem to be no seasonal differences of a regular sequence in harelip production it might be well to investigate periods of a shorter duration. Some care was exercised in studying day-to-day periods but no significant fluctuations were found in the proportions of harelip offspring produced in the shorter periods.

Feed

The only feed used was a balanced ration sold as a fox chow. Fresh water was always present. As no vegetables were given, the feeding may be considered a constant factor which would have an equal effect, if any, on all the matings.

Uterine resorption

It is conceivable that harelip embryos are absorbed *in utero* in cases where the condition might be serious enough to be lethal at early stages. Line 2 has been so derived that all animals of the last few generations are over 90 percent inbred. We find that this line is producing about 15 percent harelip in these generations, and that the average litter size is 6.8 (32 litters). This is a large litter size for mice inbred to such an extent and in which there has been no selection for litter size. It is probable that there could have been but a very small prenatal death rate of harelip zygotes after implantation in this line at least.

TABLE 7

Absence of relationship between the season of the year and the percentage of harelip

SEASON	NO. OF LITTERS	PERCENT HARELIP
December–February	75	23.3±2.6
March–May	142	24.3±2.0
June–August	122	22.0±1.8
September–November	30	30.5±4.1
December–May	217	23.9±1.6
June–November	152	23.6±1.7
March–August	264	23.2±1.4
September–February	105	25.3±2.2

The difference between the incidence of harelip in line 2 (15 percent) and in line 1 (50 percent), with the litter sizes as they are, could be explained only on some assumption other than that of a difference in resorption. A study of the embryology of harelip (REED, 1933) revealed no evidence of differential prenatal resorption of the extreme cases of harelip.

It seems improbable to the writer that there is early zygotic elimination of harelip animals. If there is zygotic elimination it may be discovered in the future if a close linkage of harelip with some "regular" character is found.

We have now investigated, within the limits of our data, the important agencies of the external environment which might influence the expression of the character. We have found no discernible effect of seasonal fluctuations, feed, birth rank, or uterine resorption. There is evidence that the condition of the mother, as measured by age, has some effect and that litter size has an effect of 3.5 percent on the total variance of harelip.

EXPRESSION OF HARELIP MODIFIED BY INTERNAL ENVIRONMENT

There were 548 males to 460 females with some type of cleft, and 272 clefts of the left side alone and 218 of the right side alone in all the harelip populations. The difference in each of the comparisons (sex and symmetry) is statistically significant.

Sex

Taking all the litters in the harelip stock in which all members were sexed at birth (undepleted litters), we find a total of 735 males to 754 females, including both harelip and normal animals. We have noticed a marked excess of males among the harelip animals, but a slight excess of females when all animals are considered; it follows that among the non-harelip there must be an excess of females. There were 412 normal females to 320 normal males. Most of these excess females are probably normal overlaps for harelip.

In human races the fact is unquestionable that harelip occurs with greater frequency in males than in females, and on the left side than on the right. This agrees with the observations on mice. In man the most serious cases are significantly more common among males than among females (SANDERS, loc. cit.) and this is perhaps true in mice. When all grades of clefts are grouped, there are 54.4 percent males; but if only the severe cases are considered there are 55.5 percent males. Further, there is an eye defect associated with the harelip in my stocks which is perhaps another type of expression of the character. It agrees in showing an excess of affected individuals of the male sex. Of the total of 203 animals with eye defects, 68 were sexed; 51 were males and only 17 were females.

The association of harelip and these eye defects is statistically significant. In the litters in which animals with eye defects appeared, there were 60 animals with both eye defects and harelip, 143 with eye defects but no harelip, 150 with no eye defects but with harelip, and 616 animals without either eye defects or harelip ($p_1 - p_2$ is equal to $.100 \pm .032$ where p_1 = percentage of eye defects in harelip animals). The eye defect is similar to that found by other workers in other strains of mice and appeared in the harelip strains. Its relation to defects reported by other workers is not known. One or both eyelids may be open at birth, often followed by considerable damage to the adult eye.

In man the usual interpretation of the sex and symmetry differences is that males are weaker before birth and that the left side develops more slowly than the right (SANDERS, 1934); therefore the excess of male and left side harelip. As we have no reason to assume that more males than females have the genetic basis for harelip, we may suggest that owing to weaker development of the male, the genotype for harelip can express itself there more often than it can in females, where the threshold is not so readily exceeded.

Cyclopia in man and lower mammals appears more frequently in females than males (WRIGHT 1934a). The threshold here is crossed more easily in females than in males whereas with harelip the threshold is more

easily exceeded in males. Presumably the two "thresholds" have quite different biological bases.

We may calculate the effect of sex on the total variance of harelip expression. In the small population (1284 animals) in which we determined the amount of variance due to age of mother, there were 166 harelip males, 476 non-harelip males, 135 harelip females and 507 non-harelip females. The harelip males constituted 25.8 percent of all the males and the harelip females 21.0 percent of all females in the small population.

We shall assume that there is a normal distribution of factor complexes underlying the dichotomy of harelip *versus* not-harelip on a scale in which the factors have additive effects. Then if σ is taken as the unit of measurement we may find the value of σ^2 after determining the means on our normal curve for males and for females. The mean of the males, and in like manner for the females, is found to be the value of the inverse probability integral of the percentage of males which are harelip minus one-half. Thus,

percent harelip (q)		$q-\frac{1}{2}$	$\text{prf}^{-1}(q-\frac{1}{2})$
♂♂	25.8	24.2	.650 = ♂ mean
♀♀	21.0	29.0	.806 = ♀ mean

Then,

$$\tfrac{1}{4}(\text{m}\,♀ - \text{m}\,♂)^2 = \sigma_{\text{sex}}{}^2$$
$$\tfrac{1}{4}(.806 - .650)^2 = .061$$

It will be recalled that the correlation *squared* between harelip and size of litter, for instance, is an indicator of the total effect of size of litter on harelip expression in the particular population which we have studied. For sex we found $\sigma^2 = .061$ so η^2 must equal 0.058. There is then a correlation, η^2, of nearly 6 percent between harelip expression and sex.

Asymmetry

The higher frequency of left clefts (55.5 percent) contrasted with right clefts (44.5 percent) brings up the problem of asymmetry.

With harelip a small portion of the asymmetry is inherited as there are significantly more clefts of the left side alone than of the right side alone. CASTLE (1906) found that a majority of his polydactylous guinea pigs were sinistral, but that there was no specificity in transmission, and his endeavor to increase the sinistrality by selection was unsuccessful.

Harelip in mice (and probably in man) behaves in just this way. From animals 90 percent inbred one can predict that, on the average, more left than right clefts will appear in their offspring, but it is found that there is no comprehensible order in the appearance of the left and right clefts. If there were particular genes for normal (or abnormal) development of the left or right side of the face, one would expect that, as inbreeding pro-

ceeds, there would be a gradual segregation so that if unilateral clefts continued to appear they would be more often on one side of the face in any inbred line. The most highly inbred lines (table 10) show no tendency for clefts of either the right or left sides alone to become established in any inbred line. The conclusion that the abnormality is inherited but that the asymmetry is not seems to be justified if we allow for the exception of the excess of left clefts.

TABLE 10

Absence of inheritance of left or right asymmetry (WRIGHT'S *coefficient of inbreeding*)

NO. OF ANIMALS IN FRATERNITY	COEFFICIENT OF INBREEDING	LEFT CLEFT	RIGHT CLEFT
	LINE 1 (IN PART)		
6	.594	0	0
39	.672	2	0
13	.734	1	0
10	.785	0	1
36	.826	3	0
10	.859	1	0
24	.886	4	1
54	.908	1	0
60	.925	0	2
50	.940	0	2
42	.951	2	1
	LINE 2 (IN PART)		
21	.785	2	0
55	.826	4	8
51	.859	3	0
85	.886	3	2
46	.908	3	2
102	.925	11	7
20	.940	0	0

There is another interesting observation on asymmetry. The eye defect associated with harelip showed an excess of affected males as did harelip itself; there is agreement as far as sex is concerned, but the asymmetry is exactly the opposite. There are more eye defects of the right side alone than of the left side, whereas with harelip the majority was on the left. There were 98 animals with the defects of the right eye, 60 with defects of the left eye and 45 with defects of both eyes.

WRIGHT'S (1934a) analysis of otocephaly shows no greater percentage of high grades of otocephaly among the more prevalent otocephalic females than among the fewer otocephalic males. With harelip we have seen that the percentage of higher grades is slightly greater (though not significantly so) among the males than among the females.

There is at least one distinction between the behavior of harelip and otocephaly. As the total frequency of harelip increases, the grade of the abnormality also increases; whereas with otocephaly, the grade of abnormality tends to decrease as the total frequency increases. In the common inbred harelip strains (high harelip frequency) 52.5 percent of the harelip animals were of the two highest grades (most severe cases), but of the various F_2 and double outcross harelip animals (low harelip frequency) only 31.5 percent were of the two highest grades. An arbitrary, but concrete, system of five grades was used.

With inbreeding the percentage of extreme cases of harelip appears to increase even though the absolute percentage of harelip of all grades may be dropping. In three harelip lines (2a, 2b, and 3) the percentage of harelip dropped with inbreeding, but the percentage of harelip animals of the two highest grades probably rises in an absolute sense (table 11).

TABLE 11

Data for lines 2a, 2b, and 3 of comparable generations

COEFFICIENT OF INBREEDING	PERCENT HARELIP	PERCENT OF TOTAL HARELIP OF 2 HIGHEST GRADES
70±	22.3	40.6
80±	15.5	74.3
90±	10.5	58.3

In line 1a, in which the percentage of harelip has been rising, the percentage of cases which were of the two highest grades has also increased (table 12).

TABLE 12

Increase of frequency and severity of harelip in line 1a

COEFFICIENT OF INBREEDING (PERCENT HOMOZYGOSIS)	PERCENT HARELIP	PERCENT OF TOTAL HARELIP OF 2 HIGHEST GRADES	NO. OF HARELIP
.734	7.7	0	1
.785	20.0	50	2
.826	26.3	60	5
.859	45.0	66	3
.886	46.0	55	11
.908	71.5	93	15
.925	59.3	100	16
.940	79.4	96	23

It might be expected that the fraternities of young among which there were the highest percentages of harelip would also show higher grades of harelip than fraternities with low percentages of harelip. This is not necessarily true because the most severe cases usually become predominant over the lower grades of harelip only after considerable inbreeding, whereas

high percentages of harelip may or may not accompany extended inbreeding. It was found that the 280 harelip born in fraternities of less than 30 percent harelip were of the lower grades in 167 instances, and of the two highest grades in 113 instances. Of the 301 harelip animals born in fraternities of over 30 percent harelip, 149 were of the lower grades and 152 of the higher grades. From the fourfold table constructed with these data, we find that $\chi^2 = 5.3$; there is one degree of freedom. There is, therefore, probably a significant association between the two highest grades of harelip and the fraternities with over 30 percent harelip.

We have found that there is no tendency for the asymmetry (side affected) to become fixed with increased inbreeding. Late inbred generations still produce left and right clefts in about the same proportion as did earlier generations. With inbreeding the variability of the expression of harelip decreases; that is, with inbreeding there are fewer unilateral clefts and more bilateral (52.5 percent extreme cases in inbred stocks but only 31.5 percent extreme cases in crossbred stocks). Though the variability of harelip expression (severeness) always seems to decrease it is clear from tables 11 and 12 and figure 1 that as the inbreeding goes on the percentage of harelip may either increase or decrease. As inbreeding progresses the character is expressed more completely and severely irrespective of the percentage of harelip.

ALLOCATION OF THE RELATIVE INFLUENCES OF ENVIRONMENTAL AND GENETIC FACTORS

WRIGHT has developed methods for determining the relative influences of heredity and environment on the variation of characters similar to harelip. It is not easy to apply some of the methods to characters which are lethal and overlap as does harelip, but the following attempts have been made. I am deeply grateful to Professor WRIGHT for advice and assistance given while the paper was being written.

The correlation between parent and offspring could be used to determine the relative influence of heredity in regard to variation of the character. Unfortunately the parents do not have visible harelip, so it is impossible to make a direct parent-offspring correlation between individual parents and offspring. It is possible to determine in a qualitative way whether or not there is some correlation between parent and offspring by finding the ordinary correlation coefficient between the percentage of harelip in the fraternity of the parent and the percentage in the fraternity of the offspring.

The correlation between the percentage of harlip in the fraternity (large population) in which the mother was born and the percentage of harelip she gave was $r = +.333 \pm .093$ while that between the percentage of harelip

in the fraternity in which the father was born and the percentage he sired was $r = +.186 \pm .104$. The average of these two correlations is $r = +.26 \pm .07$ which is fairly large and certainly significant. There is, then, a correlation between the percentage of harelip in the fraternity of the parent and the percentage in the fraternity of the offspring, though we can not use the above figures as a direct quantitative test of the amount of variance due to heredity.

What portion of the variability in our population of 1284 animals due to both heredity and environment is common to substrains? We know that the differences common to substrains, but not common to the whole small population, would be wholly genetic. It will be shown shortly that the variation common to sibships is equal to 10 percent. This *includes* the variation common to substrains, and this substrain variation should be equal to 10 percent or less.

The amount of variation common to substrains, but not to the whole population, can be calculated. The population of 1284 animals was divided into its substrains (the family tree partitioned into its branches or groups of fifty or more animals), the mean found for each substrain, $prf^{-1}(q-1/2)$, and finally biserial eta squared for the total groups. Biserial eta squared was found to be 0.109; therefore about 11 percent of the total variability is common to substrains. The 10 percent variation, common to both substrains and sibships, theoretically should include this 11 percent common to substrains. The reason for the discrepancy is that there is apparently little, if any, variability common to sibships. Otherwise the agreement is as close as could be expected.

If variation were common to sibships, it would probably be divided between genetic differences of sibships and persistent conditions of the mother. Table 3 of the following paper contains evidence that there are probably no persistent conditions of the mother which affect harelip (age of mother, etc., are not persistent conditions). We may conclude that about 11 percent of the total variation in harelip expression is common to substrains and that little if any of the total is common to sibships alone.

The determination of the 10 percent of variation common to sibships and substrains was found by comparing consecutive litters of the sibships. It has just been noted that the variation is practically all common to substrains and none to sibships. The present determination of 10 percent is useful as a check on the previous finding of 11 percent for substrains. The comparison between each individual of an earlier litter and each individual in the next litter was carried out. If there were 2 harelip and 3 non-harelip in the earlier litter and 1 harelip and 4 non-harelip in the next litter, we could form a 2×2 table such as table 8. The actual data for our small population (1284 animals) are in table 9. It is necessary to use the tetrachloric

correlation for a normal frequency surface (KELLEY 1924). The correlation was found to be $r_t = +.100$ or 10 percent.

TABLE 8

Correlations between individuals of different successive litters from the same mating

		ILLUSTRATIVE LATER LITTER		
		NOT HARELIP	HARELIP	TOTALS
Earlier Litter	Not harelip	12	3	15
	Harelip	8	2	10
	Totals	20	5	25

TABLE 9

		ACTUAL DATA LATER LITTER		
		NOT HARELIP	HARELIP	TOTALS
	Not harelip	3402	1097	4499
	Harelip	1121	485	1606
	Totals	4523	1582	6105

$r_t = +.100$

The analysis of variation common to litters but not to sibships is now possible. If we compare individuals within each litter of the small population in a manner somewhat similar to that used in comparing litters of each sibship we find that the amount of the total variation common to litter mates, and including that common to substrains, is 21 percent. We know already that the variation common to substrains is 11 percent so we may subtract this from 21 percent and get 10 percent of the total variation which is common to litters but not to sibships or substrains. The variance common to litters is divided into 4 percent due to the effect of size of litter and 6 percent miscellaneous effects as yet unaccounted for.

The variation peculiar to the individual is a result in small part of its sex (6 percent) and of any segregation within litters. The amount of segregation should be slight as all members of each litter are certainly homozygous for the main harelip genes and probably for the same set of modifiers in each litter. Our residual, the environmental factors affecting the individual, accounts for nearly 75 percent of the total variation in harelip expression of this population which is approximately homozygous. It is interesting that such intangible factors as accidents of implantation should play such an important part in the non-genetic variation of an organism.

We may conclude that the small population studied quantitatively (1,284 animals) was practically homozygous and that the variation in the expression of harelip was due in small part to sex, litter size and age of mother, etc., but in the major portion to intangible chance factors working from within the mother but not correlated with her activities. Such intangible factors might be accidents of implantation, proximity of embryos, blood supply, etc.

Stated in another way, if all members of late generations of an inbred line possess the same genotype for harelip, whether the individual will be

phenotypically harelip depends in small measure upon its sex, the litter size, the age of its mother, etc., while the main determining factors are those of accident or chance. We have no knowledge as yet just which chance factors are most effective.

Thus harelip is similar to white spotting in the guinea pig (WRIGHT 1920) in which, after homozygosis has been reached, further variation (which may be quite considerable) is due mainly to accidental influences affecting each individual more or less independently of his sibs. Such is the case with both white spotting and harelip. The similarity in behavior of harelip and of otocephaly in the guinea pig is even more pronounced.

SUMMARY

In mice there are differences in harelip expression resulting from the action of both environmental and genetic factors. Differences in harelip expression depend upon the sex of the individual, the size of the litter in which the individual was born, the age of its mother, asymmetry of the clefts, and in large part upon intangible chance factors. The variation in harelip expression resulting from these intangible chance factors may be best studied in populations in which all members are genetically harelip.

In a population of 1,284 mice from highly inbred families there was approximate homozygosis of genetic factors including those for harelip. However, non-harelip animals still appear and there is considerable variation among the animals which are phenotypically harelip. The allocation of the effects of the various factors controlling expression of harelip in these families is considered to be of about this order:

Variation common to substrains (but not common to the whole small population) Wholly genetic		11 percent
Variation common to sibships but not to substrains		0 percent
Genetic differences between sibships Persistent conditions of the mother	} little, or none	
Variation common to litters but not to sibships		10 percent
Size of litter	4 percent	
Condition of mother at a given time		
Age of mother	?	
Season	0 percent	
Miscellaneous	6 percent	
Variation peculiar to the individual		79 percent
Sex	6 percent	
Individual genetic factors (slight) (segregation within litters)		

Factors of the environment (residual)	73 percent	
Possibly Accidents of implantation		
Possibly Proximity of embryos		
Possibly Blood supply		
		100 percent

It is possible to simplify our presentation of the results in this fashion:

1. Variation due to hereditary differences		
a. Sex	6 percent	17 percent
b. Substrain	11 percent	
2. Variation due to environmental differences		
a. Litter size	4 percent	83 percent
b. Age of mother	?	
c. Miscellaneous tangible factors	6 percent	
d. Intangible factors	73 percent	

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HARELIP IN THE HOUSE MOUSE

II. MENDELIAN UNITS CONCERNED WITH HARELIP AND APPLICATION OF THE DATA TO THE HUMAN HARELIP PROBLEM

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THE results described in the previous paper and those to be presented in this paper leave no doubt that there must be more than one gene concerned with harelip expression. Whether one of these genes is necessary for any harelip expression and the others merely modifiers is not immediately apparent.

When dealing with characters which are inherited in an irregular fashion it is important to try to ascertain whether there is a single unit factor which must be present if the character is to be expressed. Whether the character is expressed or not, when the gene is present, is another matter.

An experiment was designed to test for the presence of a necessary gene. Animals of the inbred harelip stocks, some of which showed a very slight cleft and others, which were more than 90 percent inbred, were crossed with inbred non-harelip stocks. The F_1 animals were backcrossed to the non-harelip stock, and the "second outcross" animals so derived were tested as to whether they could produce harelip young by mating with a number of animals of the inbred harelip strains. All the F_1 animals used presumably carried the necessary gene or genes for harelip, because at least one of the second outcross offspring of each of the F_1 animals was found to be able to produce harelip young when tested. This assumes recessiveness of relatively few harelip genes essential for expression of the character. This assumption will be later shown to be valid.

If a single gene is necessary in the homozygous condition to produce a potentially harelip animal, then half of the second outcross animals should be able to produce harelip young and the other half not able to, when similarly tested. If we designate the essential gene as *hp*, a harelip animal would be *hphp*. The second outcross animals able to produce harelip would be *Hphp*, while those not able to do so would be *HpHp*.

Were there two or more independent genes any one of which, in the homozygous condition, could cause harelip, we should then get three or more of these second outcross animals able to produce harelip to one not able to produce it.

If one assumes two relatively equipotential cumulative factors which,

only in the homozygous condition say *aabb*, produce harelip, then only about one-fourth of the second outcross animals would be able to produce harelip.

The experiment shows that one half of the second outcross animals are able to produce harelip, and that one half are not able. The data are presented in full for each second outcross male tested. Males can be more thoroughly tested for harelip production than can females, and data from males are therefore the more reliable. Of 20 second outcross males tested, there were 10 able to produce harelip and 8 definitely not able. There were 2 animals of uncertain classification because insufficiently tested (tables 1 and 2). There are only 1.04 chances in 100 that these figures are random deviations from a three to one ratio.

TABLE 1

Offspring of the 10 second outcross males capable of producing harelip shown at the left; the young of the same test females when crossed with inbred males are shown in the extreme right column.

YOUNG HARELIP : NORMAL	PARENTS 2D OUTCROSS ♂ × TEST ♀ × INBRED ♂ (INBRED)	YOUNG HARELIP : NORMAL
	o♂ t♀ i♂	
1 : 7	4868 × 2302 × 2301	4 : 16
1 : 6	4869 × 3927 × 4402	1 : 9
1 : 6	1879 × 587 × 583	1 : 7
1 : 23	1463 × 803 × 618	1 : 12
0 : 20	1820 × 841 × 525	1 : 9
1 : 10	1820 × 1067 × 357	1 : 6
1 : 9	1820 × 623 × 622	1 : 6
0 : 45	1383 × 1045 × 1044	3 : 12
0 : 11	1383 × 1969 × 1971	8 : 11
2 : 23	1383 × 1859	
0 : 11	1383 × 1856	
0 : 5	1383 × 1858	
0 : 6	1813 × 1610 × 1609	2 : 10
0 : 8	1813 × 587 × 583	1 : 7
2 : 13	1813 × 725 × 727	3 : 5
2 : 16	1815 × 2505 × 2507	2 : 16
0 : 12	1815 × 2004 × 863	1 : 14
0 : 50	1815 × 1759 × 1611	1 : 3
0 : 15	1817 × 1366 × 1367	2 : 12
1 : 8	1817 × 1236	
1 : 8	1818 × 1253 × 594	5 : 2
	1253 × 1611	0 : 4

Unfortunately proof that fifty percent of the second outcross animals are able to produce harelip young is not conclusive proof that there is one and only one gene essential for any harelip production. We shall discuss two other possibilities which would result in a 1 : 1 ratio of producers to non-producers among the second outcross animals.

TABLE 2

Progeny tests of the 8 second outcross males not capable of producing harelip and of the 2 males insufficiently tested

YOUNG NORMAL	PARENTS 2D OUTCROSS ♂ × TEST ♀ × INBRED ♂ (INBRED)	YOUNG HARELIP : NORMAL
	o♂ t♀ i♂	
4	4998 × 5101	
34	4998 × 5103	
20	4998 × 5104	
58		
29	5000 × 2938 × 2939	8 : 20
64	5000 × 6181 (a daughter of 2938 × 2939)	
93		
10	6343 × 2302 × 2301	4 : 16
14	6343 × 2303 × 2301	4 : 15
33	6343 × 4727	
23	6343 × 4728 (daughters of 2302 × 2301)	
4	6343 × 4726	
84		
23	6364 × 3927 × 4402	1 : 9
26	6364 × 6512 (daughters of 3927 × 4402)	
27	6364 × 6514	
76		
25	1878 × 2399 × 2367	1 : 7
9	1878 × 1969 × 1971	8 : 11
11	1878 × 1333 × 1117	10 : 6
18	1878 × 1859 × crossbred	2 : 23
26	1878 × 1320 × 1436	5 : 11
33	1878 × 2396	
25	1878 × 2397 (daughters of 1320 × 1436)	
25	1878 × 2398	
20	1878 × 1856	
10	1878 × 1858	
202		
19	1379 × 1045 × 1044	3 : 12
7	1379 × 711 × 710	1 : 2
19	1379 × 2364 × 2363	3 : 24
30	1379 × 2412 × 2411	8 : 5
25	1379 × 2413 × 2411	5 : 14
25	1379 × 3330 × 3333	7 : 8
17	1379 × C17 × C13	4 : 1
142		

TABLE 2 (*Continued*)

25	2171×1799	
16	2171×1393×1394	6:8
25	2171×1396×1394	12:21
10	2171×2505×2507	2:16
13	2171×2937×2939	5:8
89		
30	1819×848 ×845	4:3
	848×583	4:6
25	1819×1902	
16	1819×3328×3333	9:14
17	1819×3329×3333	2:13
19	1819×3331×3333	8:10
55	1819×2545	
27	1819×2546	
189		
7	4867×2396	
9	4867×2397 (daughters of 1320×1436)	
2	4867×2398	
4	4867×2399 ×2367	1:7
22		
11	6362×3328×3333	9:14
6	6362×3329×3333	2:13
17		

Considering two factors (harelip strain *aabb*, normal strain *AABB*) the second outcross generation would consist of *AaBb*, *AABb*, *AaBB* and *AABB*. The first class (*AaBb*) might be expected to produce harelip frequently (when crossed to inbred harelip strains assumed to be *aabb*) the next two classes might produce harelip so rarely that even with large numbers from each animal only about half would produce any harelip while the last class (*AABB*) would produce none. In this case the observed 1:1 ratio would be accidental.

The second possibility is that three equal factors are involved of which any two (homozygous) are required to give a chance of harelip. Fifty percent of the second outcross individuals would then be producers (*AaBbCc*, *AaBbCC*, *AaBBCc*, *AABbCc*) while the other fifty percent would be nonproducers (*AaBBCC*, *AABbCC*, *AABBCc*, *AABBCC*). Five equal factors of which any three are necessary would give a similar result.

Presumably the number of factors necessary for harelip production is not very large as shown by the pronounced segregation in the F_2 with 6.4 percent harelip (table 3).

Environmental, and in particular developmental, factors have been shown to be of importance in the normal overlapping of the character in an inbred line (REED 1936). Much overlapping of harelip in heterogeneous populations may be caused by genetic modifiers, however.

TABLE 3

Various reciprocal crosses demonstrating the genetic basis for harelip

FATHERS	MOTHERS	NORMAL YOUNG	HARELIP YOUNG	PERCENT HARELIP YOUNG
Crossbred *Hphp* (F_1)	Crossbred *Hphp* (F_1)	513	35	6.4
Crossbred *Hphp* (F_1)	Inbred stocks	226	37	14.1
Crossbred *Hphp* (2nd outcross)	Inbred stocks	312	14	4.3
Inbred stocks	Crossbred *Hphp* (F_1)	188	28	13.0
Inbred stocks	Crossbred *Hphp* (2nd outcross)	336	17	4.8
Inbred stocks	Inbred stocks	2382	611	20.4

The analysis in the previous paper demonstrated some of the factors which cause genetically harelip animals to be phenotypically normal. In addition a few animals with visible clefts were raised to maturity. The normals from inter se matings of these animals may be normal overlaps; six of the sixteen young from parents with visible clefts were phenotypically non-harelip. CLOUDMAN (1932) apparently had a similar experience in crossing viable harelip of his stock (all parents showing clefts).

Table 3 demonstrates the expression of harelip in crosses allowing extensive genetic segregation. If we assume that the contribution of the father is almost entirely chromosomal, then by mating different kinds of males to the same genetic type of females the variations in the percentages of harelip reflect the genotypes of the fathers.

The genetic differences in the fathers are clearly shown. Crossbred fathers, if F_1 animals, are able to produce about 14.1 percent harelip young when mated with inbred females. If the crossbred fathers are from a second outcross to normal, and produce harelip at all, they produce only 4.3 percent harelip when mated with similar inbred females. The two groups of inbred females would not differ essentially in the aggregate, but there is no doubt that there is a genetic difference between the two kinds of crossbred males. Incidentally, the 4.3 percent shown above is too high because six of the ten second outcross males were not tested further after having produced their first harelip young.

If there is a single essential gene, *hp*, for harelip expression, the difference between the two kinds of males must be due largely to genetic modifiers. A difference in the number of factors, which can produce harelip, that are present in the two types of males is indicated if there are cumulative genes for harelip expression no one of which by itself (when homozygous) can produce harelip.

It is apparent, when one observes the reciprocal crosses, that there are no tangible uterine or other maternal factors of great importance influencing the percentages of harelip, and that the constitution of the zygote is the important factor in determining the differences of harelip frequencies in these crosses. The crossbred females (both F_1 and second outcross) gave 45 harelip and 524 normal (7.9 percent) when mated with inbred males. Crossbred males of both types gave 51 harelip and 538 normal (8.7 percent) when mated with inbred females. This important point is clearly shown in table 3. The inbred females, supposedly weaker and perhaps less fertile, do not produce significantly more harelip than the rugged second outcross females when the genetic constitutions of the young produced are the same. The backcross data alone are of even more significance than when combined with the second outcross data. Inbred females by F_1 males produced 14.1 percent harelip while F_1 females produced 13.0 percent harelip by inbred males; there is no significant difference between the two percentages. This type of reciprocal cross, which gives genetically identical groups of young borne by two very different genetic classes of mothers, allows one to show clearly that uterine influences and vigor of the mother in the usual tangible sense are of little account.

Ten second outcross males produced 14 harelip and 312 normal or 4.3 percent harelip. As previously noted this percentage is somewhat too high as six of the ten males were not tested further after producing one harelip young. The males that never produced harelip young were tested more extensively, the ten of them producing 972 normal young. Eight second outcross females produced 17 harelip and 336 normal or 4.8 percent. All these females were tested as long as they were fertile and averaged 2 harelip and 42 normal each. The 7 females that never produced harelip but which gave 23 to 72 normals produced 287 normal in all, an average of 41 normal.

Among highly inbred harelip strains one line may show as low as 20–40 percent overlapping of harelip with the normal, while another line may show nearly 100 percent overlapping. The percentage of overlapping in the backcross of F_1 animals to the inbred harelip strains is 72.8. The overlapping of harelip in the F_2 generation is 74.5 percent. When the second outcross animals are crossed with the inbred harelip strains there is 91 percent overlapping. This increase in percentage of overlapping in the young from second outcross animals is surprisingly large, if assumed due to one factor.

Table 3 shows a major genetic basis controlling the expression of harelip. We now have a choice of two genetic systems which could be involved. We may decide that a few important genes determine the percentage incidence in a strain, while chance differences are decisive in regard to litters, sibships, and individuals. The other prominent alternative is that there is fixation of an essential gene for harelip (*hp*) followed by fixation of some system of modifiers which determine the percentage incidence in a strain; chance factors are decisive after the fixation.

Under either genetic system the lines could continue indefinitely with the production of about the same percentage of harelip in each generation (barring new mutations).

Table 3 gives evidence in favor of the hypothesis of a small number of cumulative genes which permit harelip production, in distinction to that of an essential gene, *hp*, with genetic modifiers. We found 6.4 percent harelip in the F_2 which indicates that 25.5 percent of the genetically harelip show clefts (74.5 percent overlapping). The overlapping in the F_2 is no greater than in the inbred stocks as a whole, however, for only 20.4 percent of the supposedly genetically harelip showed clefts in the whole group of inbred populations. On the one factor hypothesis this indicates that the modifiers introduced from the outcrossing to get the F_2 were as favorable to harelip as those of the inbred stock. Again, the backcross of F_1 to harelip produced 13–14 percent harelip indicating that about 27 percent of those genetically harelip (on the essential gene hypothesis) showed clefts. But the second outcross animals which were producers produced only 4 or 5 percent harelip on mating to *inbred* harelip stocks, indicating (on the essential gene hypothesis) that only about 9 percent of those genetically harelip show clefts. These young showing such a small percentage of clefts (9 percent equals 91 percent overlapping), have however, 5/8 of the blood of the inbred harelip strain and thus should have more modifiers favorable to harelip than are found in the F_2 which has only 1/4 of the blood of the inbred harelip strain.

This suggests some multiple factor scheme (a few cumulative genes no one of which by itself, when homozygous, is sufficient to produce harelip) and is contrary to the expectations on the hypothesis that there is one gene essential for harelip production, and only one, augmented by modifiers.

Harelip has been found in three laboratories. Its discovery has been reported by CLOUDMAN (1932) by REED and SNELL, while PINCUS, of the Harvard Biological Institute, also finds it in his albino stock. It is interesting that it has been found in all three cases in strains of albino mice all of which descended from the well known Bagg albinos. This may be evidence that the original albino strain was homozygous for the essential hare-

lip gene and protective modifiers. It may have been as a result of replacement of these protective modifiers by mutation, chromosomal changes, outcrossing, or other methods through many generations, that these investigators were able to detect the basic harelip gene, *hp*, if such exists.

CLOUDMAN (1932) has reported that when his harelip stock is crossed with a certain non-agouti strain which has been inbred in the JACKSON laboratory a low percentage (1–3 percent) of harelip young are produced in F_1. In the many crosses with a large number of normal strains which the writer has made, harelip has never been observed in F_1. I assume, therefore, that the Little non-agouti strain differs a great deal genetically in regard to harelip from the other strains of normal mice with which I have worked. The writer suggests that if there is a single harelip gene the Jackson laboratory non-agouti strain is homozygous for it, as has been suggested for the various Bagg albino strains, but that it is also homozygous for a modifier complex which completely prevents expression of the gene in the non-agouti stock. This hypothesis is now being tested.

The effect of heterosis upon harelip should be of interest. We should not expect to find much effect, as we know from table 3 that the greater vigor of the double outcross females did not affect the expression of harelip in the embryo. Animals of my inbred strains were crossed with those from the highly inbred strain developed by Professor PINCUS and found to produce harelip. I wish to acknowledge his kindness in supplying me with his strain on two occasions. There were produced from this cross between the two highly inbred strains 20 (17.4 percent) harelip and 95 normal young. The parents from the PINCUS stock had produced 14.6 percent harelip before being used in the heterosis experiment, while the parents from my stock were from fraternities which averaged about 17.5 percent harelip. Both parental strains and the hybrid were of approximately the same genetic constitution.

Heterosis presumably produced more vigorous offspring, and if vigor of the offspring had an effect on harelip, there should have been either a higher or lower percentage of harelip instead of the percentage observed. Consideration of the above data supports the conclusion that hybrid vigor has no effect on the percentage of harelip. The following cases, in which there was a reduction of harelip percentage after crossing two distinct harelip lines may be considered to be the result of heterozygosis of various specific genetic modifiers of harelip, or of cumulative genes, and not due to increase of physical vigor.

Perhaps one should look for an effect of modifier recombinations in the next generation after the outcross. ♀ 1369 of my stock, crossed with ♂C7 of the PINCUS stock, produced 7 harelip and 7 normal young in the outcross generation; 3 of the 7 normal were mated and produced 52 normal

offspring but no harelip. Still another generation (the next) seems to have lost the modifier heterozygosis to some extent, for when 2 of the 52 normal were crossed, 2 harelip and 18 normal were produced.

When lines 1 and 2 of my stock were crossed there seems to have been an immediate heterozygosis effect for 31 harelip to 275 normal were produced, a considerably lower percentage of harelip than that of either line 1 or line 2.

New harelip incidences could be expected in the hybrid strains owing to the segregation of modifier combinations different from the combinations of the parent lines.

The study of the assumed modifiers would be greatly assisted if an essential gene for harelip were found to be closely linked with some other gene determining coat color or hair texture. A closely linked coat color gene would serve as an excellent marker for the harelip gene whether the harelip is expressed or not. Further still, if a linkage were found it would settle once and for all whether there is one, and only one, gene capable of producing harelip if in the homozygous condition and accompanied by the proper modifiers. With this in mind attempts were made to discover a close linkage.

LINKAGE

The difficulties of linkage tests are many when the character overlaps normal and is lethal soon after birth. It is obvious that tests for loose linkages would not be successful, but it was considered worth while to search for a close linkage. The tests were carried out in numerous ways, not all of which constitute usual linkage procedures.

Harelip can be tested most satisfactorily with characters such as brachyury which are dominant and can be scored at birth. If a brachyuric animal is crossed with a genetically harelip, normal tailed animal and the brachyuric F_1 animal is then backcrossed to the harelip parent, such harelip individuals as appear among the offspring should be equally divided between brachyuric and normal-tailed groups if there is independent assortment; with close linkage few brachyuric harelip animals would appear. In consideration of the excess of crossover gametes (table 4), it appears that there is little probability of even a loose linkage between harelip and brachyury.

Genes which can be classified at birth only when homozygous are also satisfactory for studying possible linkage of harelip. The absence of linkage of harelip with members of this class of characters is also shown in table 4.

Unfortunately there are several characters which can not be classified until several days after birth. This caused great difficulty in this experiment, because the harelip animal dies of starvation before it can be classi-

fied for the character for which a linkage test is desired. Because of this situation, it became necessary to test animals of a second backcross generation for the presence of the harelip gene in the heterozygous condition.

The eye color, pink, was tested with harelip in this fashion. The F_1 from a cross of a pink-eyed, non-harelip by a non-pink-eye harelip is found to carry both-pink-eye and harelip in the heterozygous condition. This F_1 is backcrossed to the pink-eye non-harelip parent, and four genetic types of offspring are produced: *ppHpHp*, *PpHphp*, *ppHphp* and *PpHpHp*. The last two classes each contain a crossover gamete and would not appear if pink-eye and harelip are inseparably linked.

TABLE 4

Absence of a close linkage between harelip and the characters Naked, Clark's anemia, albinism and Brachyury

	CROSSOVER GAMETES	PARENTAL GAMETES	RATIO
	NAKED HARELIP	NON-NAKED HARELIP	
Observed	8	10	
Expected	4.5	13.5	1:3
	CLARK'S ANEMIA HARELIP	NON-ANEMIC HARELIP	
Observed	2	6	
Expected	2	6	1:3
	NON-ALBINO HARELIP	ALBINO HARELIP	
Observed	18	11	
Expected	14.5	14.5	1:1
	BRACHYURIC HARELIP	NON-BRACHYURIC HARELIP	
Observed	20	7	
Expected	13.5	13.5	1:1

It is obvious that in order to tell whether any of these four classes are homozygous or heterozygous for non-harelip, each animal must be raised and tested to determine whether it carries harelip or not. This is a laborious task, for, as we have seen in tables 1 and 2 the double outcross animals only produce about 1 percent harelip young even when mated with the inbred stocks. No animal was considered to be homozygous for non-harelip until he had produced at least 30 normal young. The inbred females used in testing were those of tables 1 and 2 while the linkage results are given in table 5.

It is well known that albinism and pink-eye are linked, and we can be fairly certain that the harelip gene (if there is a single essential one) does not lie on the albino-pink-eye chromosome, as no linkage with harelip was evidenced by either character. Combining the data for both albinism and pink-eye, there are 30 crossover to 27 parental type gametes.

In summary, no close linkage exists between harelip and these characters: albinism, dilution, brown, brachyury, Clark anemia, naked. Linkage experiments with other characters and harelip are in progress.

There is probably a statistically significant excess of brachyuric harelip

animals shown in table 4. If this excess is actually significant we may assume that harelip overlaps less frequently when in an animal heterozygous for brachyury than it does in other mice. It is hoped to investigate the possible physiological or genetic effects of brachyury upon harelip in another study.

TABLE 5

Absence of close linkage between harelip and the genes for pink-eye, brown and dilution

	CROSSOVER GAMETES		PARENTAL GAMETES	
	PpHpHp (3)	*ppHpHp* (9)	*PpHphp* (8)	*ppHpHp* (8)
Observed	12		16	
Expected	14		14	
	BbHphp (2)	*bbHpHp* (1)	*BbHpHp* (3)	*bbHphp* (0)
Observed	3		3	
Expected	3		3	
	DdHpHp (5)	*ddHphp* (14)	*DdHphp* (3)	*ddHpHp* (6)
Observed	19		9	
Expected	14		14	

APPLICATION OF THE MOUSE DATA TO THE HUMAN HARELIP PROBLEM

Though the investigation of the inheritance of harelip in mice is not yet complete, enough has been learned to enable us to discuss profitably the analogous condition in man in the light of this study.

Many writers have considered harelip in man to be a dominant mutation. In a recent publication, SANDERS (1934) has shown very clearly that there is little evidence in human material that harelip ever acts as a dominant. It is true that the old pedigrees in the literature give a strong impression of dominance, but these old pedigrees were presented merely as evidence of the hereditary basis of harelip and not as support of any particular type of Mendelian system. It was only the families (in the old literature) which were afflicted with a high harelip frequency which ever got into the journals, and few normal members of these families were included.

Both BIRKENFELD (1926) and SCHRÖDER (1931) found a few families in which harelip seemed to behave as a dominant. It was pointed out that the cases thought to be dominant were the most severe in expression. These could probably be explained if the supposedly normal parent was heterozygous for harelip or was even a normal overlap for harelip and thus genetically homozygous. It will be recalled that mice of the line 1a gave from 40–75 percent harelip although none of the parents showed clefts. One mating of two phenotypically normal animals produced 23 severe harelip to 6 normal young. SANDERS calculates that one out of every 16 persons near Rotterdam must carry harelip; thus the chance of an affected harelip person mating with a normal overlap is not to be neglected.

The most recent extensive study of harelip in man has been most satis-

factory, in that it includes practically all the harelip born in a certain part of Holland within the last twenty years. SANDERS (1934) has traced his pedigrees back for seventeen generations in some cases and has connected affected persons to a previously unrecognized common ancestor. In a small town near Rotterdam considerable inbreeding has taken place with an interesting increase in harelip frequency.

It is now well established that in both the mouse and human material there is an excess of harelip males over harelip females and of clefts of the left side compared with the right. These two facts tempt the investigator to go astray in his interpretation of the genetics of harelip.

SANDERS postulates five cumulative recessive factors necessary to produce harelip and cleft palate of the most severe degree. However, he considers that one of the factors is related specifically to the left side of the lip, another to the left side of the palate, and so on. I think SANDERS will agree that the cumulative genes control the extent or intensity of the physiological actions which result in harelip, in the sense that the more harelip genes present the greater the physiological effect and hence the more extensive the clefts. Thus the cumulative genes would be concerned with increasing or decreasing the whole process rather than with affecting a particular part of the lip to the exclusion of a part of the palate.

The above seems to agree with the case in mice. There we postulated either a single essential gene for harelip with modifiers or a few relatively equipotential cumulative genes (say 3–5). It was noted that with inbreeding it was impossible to fix any genes affecting the left side in contrast to the right side.

FORTUYN (1935) criticizes SANDER'S idea of genes having specific effects on one side of the lip in contrast to the other but falls into the other error concerned with the excess of harelip males. He thinks that there are twice as many males with harelip as females (61.29 ± 2.48 of BIRKENFELD and 63.52 ± 2.42 of SANDERS). These percentages are not particularly close to 66.66 percent males in either case, it seems to me, as the deviations are nearly significant; in 1,008 harelip mice 54.4 percent were males. In mice it is obvious that there is an excess of males but it is just as clear that there are not twice as many males as females.

FORTUYN makes use of the excess of harelip males to advance a duplicate gene hypothesis to explain the genetics of human harelip. He considers that harelip and cleft palate are inherited in man as a double recessive trait, one gene lying in the sex chromosome and the other gene in one of the autosomes. If harelip is a double recessive trait, only women with the genotype *aabb* and men with the genotype *aab*-will have harelip. FORTUYN seems to think that there should be twice as many men as women with harelip if *b* is sex-linked; this would be true only by accident.

FORTUYN collected data from SANDERS and others and then selected families which agreed with his hypothesis. It was easy to pick families in which the harelip children were of one sex only, because usually there was only one harelip child in each family. There were only 537 harelip offspring in the 467 families which contained harelip children, an average of 1.15 harelip per family.

On the scheme FORTUYN describes one must expect more harelip sons from the mating of harelip mothers by normal fathers than from the mating of harelip fathers by normal mothers. His table I shows this excess of harelip sons from harelip mothers by normal fathers; this theoretical excess does not actually materialize, however. FORTUYN'S table X shows that there were 17 harelip sons observed from the mating of harelip mothers by normal fathers and 15 harelip sons from the mating of harelip fathers by normal mothers. Obviously 17 is not significantly greater than 15 allowing for any kind of reasonable error. FORTUYN overlooked this crucial test which proves that the excess of harelip males observed by so many workers is independent of sex linkage.

Presumably the excess of harelip males in men and mice is due to the effect upon harelip expression of physiological differences between males and females (greater normal overlapping of females) and is not the result of a particular gene of major importance for harelip which might be on the sex chromosome.

In view of the very striking correspondence of the genetic mechanisms in the inheritance of the parallel harelip mutations in men and mice, it would seem that the data from the mouse experiments should be directly applicable to the human problem. If, in future experiments, a method is found whereby the environment (external or internal) could be so regulated as to reduce harelip frequency in an inbred strain of mice we could predict that probably similar environmental control in man, if practical, would achieve a similar result.

I wish to express my gratitude to PROFESSOR W. E. CASTLE who has provided moral and material support throughout the rather long period of these experiments.

SUMMARY

Records of some 11,000 animals have been available for this analysis of the inheritance of harelip in mice. It is probable that harelip results when a small number of cumulative genes are present in the homozygous condition; provided that chance and environmental conditions are favorable. 6.4 percent of harelip animals are recovered in the F_2 generation which thus limits the number of chief factor pairs concerned. The other probable hypothesis is that there is one gene which when homozygous allows harelip

expression, the extent of which is controlled by many genetic and environmental modifiers.

When the genetic basis for harelip is present the phenotypic expression of harelip may be prevented in nearly all to practically none of the genetically harelip animals. Such normal overlaps depend upon various chance and environmental circumstances.

The inheritance of harelip in man is strikingly similar to that in mice; this allows us to conclude that probably the genetic and environmental mechanisms involved are nearly identical.

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HETEROSIS

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INTRODUCTION

THE fact that vigorous growth so frequently accompanies hybridization has puzzled biologists for nearly two centuries. The phenomenon was given a purely formal solution some twenty-five years ago when a series of analyses of the effects of inbreeding in maize, supplemented by studies of the results obtained when these inbred lines were crossed, made it possible to regard heterosis as something incident to the operation of genetic laws. Nevertheless, the picture of these laws in action was extremely vague. Genetic knowledge, at the time, was so meagre that it seemed necessary to assume that vigor is promoted when the genes at certain loci are unlike,—an assumption for which there was no proof, and which was not illuminating as a dynamic interpretation.

Somewhat later JONES (1917) suggested that this postulate was unnecessary. Owing to the establishment of MORGAN's theory of linkage, it became possible to see how heterosis could result from normal gene action and yet be a phenomenon accompanying hybridity. The essential points of the argument are as follows. Genes affecting growth are found at numerous loci and have cumulative action. These genes mutate frequently to more efficient (dominant) allelomorphs, and to less efficient (recessive) allelomorphs. Since any given chromosome may contain x dominant genes and y recessive genes distributed more or less at random, it follows that heterosis will be manifested very frequently in F_1 hybrids, owing to the apposition of dominant to recessive at various loci, and that the recovery of homozygotes having the characteristics of the individuals of the F_1 generation will be virtually impossible, owing to the rarity with which the crossovers required to produce homozygosis may be expected to occur. BRUCE, and KEEBLE and PELLEW (1910) are sometimes given prior credit for this conception, but not upon just grounds. The earlier authors based their theory upon independent segregation at a time when linkage was not understood, and their scheme did not and could not fit even the then known facts. The particular use made of the notion that the chromosomes are strings of genes obeying special laws of transfer was what made JONES' theory acceptable, not simply the employment of the words *dominant* and *recessive*.

The explanation of heterosis offered by JONES was so probable that it was generally accepted, at least until 1930 (ASHBY), in spite of the fact that there was no direct proof for it. This was not altogether fortunate.

Even though there is, as I believe, much critical evidence in favor of the essential features of JONES' ideas, the subject should not be regarded as a closed issue. Certain ramifications are still puzzling.

THE PHYSIOLOGICAL THEORY OF ASHBY

Numerous items of fact on heterosis have been published since 1918, but the only attack on the theoretical aspects of the problem has been that of ASHBY (1930, 1932).

The inspiration of ASHBY's investigation was a statement by V. H. BLACKMAN that the two major factors determining the amount of tissue produced by a plant are the size of the seed (or embryo) and the efficiency index (that is, the angle of slope of the logarithmic curve of growth). ASHBY divides the second factor into two components,—the length of time that the efficiency index remains constant, and the form of the growth curve after that time. This declaration, which is a restatement of the problem rather than an explanation, ASHBY regards as an analysis. He says that the next step is to discover the way in which all these factors are inherited. Undeterred by its length or the obstacles involved, ASHBY proposed to take this step.

Maize was selected as the experimental material. Three ears were obtained from the Bureau of Plant Industry, viz., inbred P_w, inbred P_b, and the F_1 from $P_w \times P_b$. The seedlings were thinned to 1 per hill, and 10-plant samples were gathered fortnightly during 16 weeks, from which to calculate leaf area and dry weight. From these data, growth curves were constructed by plotting the logarithms of the figures against time. The curves proved to be straight lines; and the curve for the F_1 was approximately parallel with that for P_b, though higher. In addition, the average dry weight of the embryos was determined to be .038 gm., .0125 gm., and .045 gm., respectively, for P_w, P_b, and F_1; while the dry weights of the seeds were .368 gm., .163 gm., and .405 gm., in the same order. Cell size was also examined.

ASHBY concluded: (a) that the hybrid does not differ in the least from its more vigorous parent as regards relative growth rate, or from either parent as regards cell size, photosynthetic efficiency of leaves, or the time of flattening of the sigmoid growth curve; (b) that the only physiological differences observed are an increased percentage of germination of the hybrid seeds and a greater initial weight of embryo which gives an advantage that is maintained throughout the "grand" period of growth; and (c) that the relative growth rate is apparently inherited in the manner of a dominant Mendelian factor.

One of the major omissions in this first work was the test of reciprocal crosses. It was rectified in ASHBY's second paper. This later contribution

also contained calculations of respiration rate. The conclusions of the previous paper were thought to have been corroborated. Reciprocal crosses had the same efficiency index but showed different degrees of hybrid vigor that were attributed to variation in embryo weight. Thus it follows, according to ASHBY, that "hybrid vigour in these strains is nothing more than the maintenance of an initial advantage in embryo size." And he believes that the notion that the higher of the two parental efficiency indices behaves as a "factor" dominant over the other, is also corroborated.

It was hardly to be expected that geneticists should share ASHBY's complete confidence in his solution of such an ancient and involved problem. Nevertheless, in spite of the meagre evidence that he has presented, his obvious lack of acquaintance with manifestations of heterosis, his failure to consider adequately the pertinent published evidence on the subject, and his unorthodox expressions when dealing with genetic concepts, ASHBY's papers should not be overlooked, for one group of his observations is very much worth while.

Let us examine first the question of the influence of the size of the embryo—or of the endosperm—on heterosis. Presumably no one would argue that there is any high degree of correlation between seed size and rapidity of growth when different groups of plants are compared, since numerous small-seeded plants produce tissue at a faster rate than do seedlings from large seeds like those of *Cocos nucifera*. But within the species, and often within the genus, the size of the seed is undoubtedly a growth factor. The phenomenon has been noted many times. In maize alone HAYES and GARBER (1927) cite five authors who have considered the matter.

It is commonly assumed that the F_1 seeds in maize hybrids are larger than those of the maternal parent, and that both the endosperm and the embryo are increased in size. In fact, ASHBY's own data show that this is true, despite his emphasis on embryo only. EAST and JONES (1920, table 11) reported the weights of seeds in a series of reciprocal crosses, using the same parental individuals. The average seed weight of parents from A stock was 24.2 cg., and from B stock was 24.4 cg. The average weight of the seeds from the A×B crosses was 28.0 cg, while that from the B×A crosses was 30.2 cg. Thus there was an increase over the seed parent of 15.3 percent in the first instance, and of 24.2 percent in the second. A smaller series (5 reciprocals) was submitted (table 15) where embryos, pericarps, and endosperms were isolated and weighed separately. The embryos were materially larger than those of the larger parent in only one case, but the endosperms were more than one-fourth larger in every case. There are also a few data on the yields from reciprocal crosses made from the same stocks used in determining the effect of crossing on seed size

(table 25). The great increases observed in each case seem hardly attributable to seed size alone.

There are a number of similar observations on other species in the literature, though there is none, so far as I am aware, where the component parts of the seed have been separated. The evidence is contradictory. Some times the F_1 seeds are larger than the seed parent; sometimes they are not larger than the arithmetic mean or the geometric mean between them. MALINOWSKI (1935), for example, crossed two varieties of the common bean weighing, on the average, .54 gm and .32 gm, respectively. The F_1 average weight was .42 gm, which is slightly closer to the geometric mean (.416) than to the arithmetic mean (.43). The plants themselves exhibited marked heterosis. Some years ago, I crossed two varieties of peas together that had seeds of practically the same size. The F_1 seeds were not significantly different, yet the plants showed a high degree of heterosis. Again, I have crossed *Nicotiana Tabacum* and *N. rustica*, obtaining plants showing more heterosis than any other crosses I have ever observed; yet no difference in seed weight could be detected.

I am very much inclined to believe, therefore, that there is no general rule about increase of size in either the endosperm or the embryo in the seeds of crosses, although heterosis is so generally to be noted in the plants themselves. Certainly MALINOWSKI's and EAST's observations on legume seeds, where endosperm tissue is virtually non-existent, do not favor the idea that F_1 increases in embryo are necessarily common or important. And in the experience of EAST and JONES on maize, the increase in the endosperm is often materially greater than the increase in the embryo; while in the experience of the wheat breeders, certain crosses between tetraploid and hexaploid types exhibit noticeable increases in vigor, even though the endosperms are shrivelled and the embryos are no larger than those of the parents. It seems reasonable to conclude, therefore, that increase in endosperm or embryo size is itself a phenomenon of heterosis detectable, at times, in early stages of ontogeny. Moreover, where considerable increase in size of endosperm appears, as in certain crosses of maize, and the amount of difference between reciprocals is significant, the latter may be a factor of some account to the end of the life cycle, thus helping to explain the differences in yield of reciprocals sometimes observed.

There is other evidence that hybrid vigor can not be "nothing more than the maintenance of an initial advantage in embryo size." JONES (1918, fig. 3) studied the growth curves of maize as exemplified by height, and their end points as illustrated by yield of grain, for parental stocks, F_1 populations, and F_2 populations. The data for the inbred parental stocks and for the F_1 generation may be taken as representative of any

given plant, for the dispersion coefficient is small. The data for the F_2 population express an average where the dispersion coefficient is large. The plants of the inbred parental stocks show less vigor than the plants of the F_1 or F_2 populations. The plants of the F_1 generation, owing partly, no doubt, to the larger seeds from which they come, grow faster than either parent, reach sexual maturity earlier in most cases, and gain a greater total height and weight. The F_2 seeds borne on the F_1 plants, however, are *larger* than the F_1 seeds borne on the parental plants; yet the F_2 seedlings continue to grow faster than the F_1 seedlings, through their initial advantage, *only* up to about 70 days from planting; after which they grow more slowly and fail to reach the height of the preceding generation.

Two direct tests of this point have been made since the publication of ASHBY'S papers, moreover, and the results have failed to sustain his position. In COLLINS' laboratory (RICHEY 1935) it was found that the larger hybrid seeds of maize had a significantly higher growth rate for only two weeks, after which no difference was detectable. In Miss PASSMORE'S (1934) experiments on reciprocal cucurbit crosses it was found that, while the cross having the larger seeds reaches its ultimate size before the reciprocal, the reciprocal catches up by having a longer period of growth.

These experiments are sufficient, in themselves, to show that ASHBY failed to get to the heart of this part of the problem; and they are corroborated, as far as I can learn, by the more casual observations of other workers on a variety of species. At all events, they are supported by my own observations. I have seen several Nicotiana hybrids, for example, that were double the height and bulk of either parent, although there was no detectable difference in the size (weight) of selfed and of crossed seeds on the maternal parent. The conclusions indicated, it seems to me, are: (1) that seed size is a nutritional advantage, other things being equal; and (2) that it may be one of the manifestations of heterosis or may be the result of other causes, but that seed size, or the size of any part of the seed, can not be the true cause of heterosis.

There is also much other evidence, of a somewhat different character, destructive to this particular thesis; so much, in fact, that one hardly knows what to select. One might cite the direct test of the hypothesis made by LINDSTROM (1935) on maize, where the handicap of the F_1 plants in "initial capital" was reduced by cutting back the seedlings. The F_1 plants gave much higher yields of grain than the inbred parents did, despite the "initial capital" reduction and the mutilation. Since animal crosses also show heterosis, one might cite the experiment of ROBERTS and LAIBLE (1925), where a Duroc Jersey sow was double mated to both Duroc Jersey and Poland China boars. Ten pigs were born. Six were pure Duroc Jersey and four were crossbred. They were distinguishable by color char-

acters. The crossbreds averaged half a pound more than the purebreds at birth; while at six months the two purebreds still alive weighed 183 and 188 pounds, respectively, and the crossbreds weighed 224, 225, 231, and 261 pounds, respectively. Other experiments with crossbred animals are not quite so critical as this one,—for example LIVESAY (1930) on rats; but in many of them marked heterosis is noted and recorded. Just how cross-fertilized animal eggs could show more "initial capital" than line-fertilized eggs is difficult to imagine. And beyond the special experimental data on the matter, there is a body of general information that appears to destroy ASHBY's hypothesis utterly. Nearly all our asexually propagated horticultural plants derive the vigor that has made them useful from heterosis, since they invariably lose much of this vigor when inbred. Among them may be mentioned potatoes, apples, pears, peaches, grapes, strawberries, raspberries, etc. *And it is well known that these plants hold their heterosis not only through a single life cycle, but also through a series of life cycles.* Deterioration of asexually propagated forms does sometimes occur, but it has been established that this phenomenon is due to disease and not to asexual propagation in itself or to loss of heterosis. This is shown by the fact that most clones of our various asexually propagated fruits and vegetables retain their characteristics over long periods, though a few may degenerate. Disease is demonstrable in many of the degenerate stocks.

Heterosis, therefore, is not simply a manifestation of an initial advantage in embryo size or the size of any other part of the seed or the propagating piece. It is a genetic effect on the organism as a whole, as has been suggested many times, and as even ASHBY's mathematical treatment of his results has demonstrated; but it is an effect controlled by many genes and is not interpretable as a simple Mendelian dominant. The last statement is a fact well known to all plant breeders who have had occasion to cross a series of strains on one particular strain, and it needs no extended defense. What I shall endeavor to do in the remainder of this paper is to show that JONES' hypothesis is not a dominance hypothesis, as it is usually called, but is instead a linkage hypothesis that is strongly supported by the evidence from amphidiploids. At the same time I shall hope to show that a modernized conception of gene action leads us back closer to the original heterozygosis theory.

THE DIRECT EVIDENCE ON THE LINKED GENE THEORY

The recent work on the salivary gland chromosomes of Drosophila and other dipterans indicates that these organisms possess several thousand genes. Presumably the number in most other organisms is greater rather than less. It need not follow that the number of *different* allelomorphs in a

population of any given strain is high; but more and more evidence accrues daily that these also are to be numbered in the hundreds. It can readily be seen, therefore, that if developmental vigor is dependent upon any very large number of genes existing in various allelomorphic conditions, the chance of obtaining homozygous forms like those appearing in the F_1 generation is very low, even with free recombination. With a relatively small number of linkage groups and such distribution of essential genes as is actually found (see LINDSTROM 1920, for chlorophyll development in maize), the chance would ordinarily approach zero. Yet a significant degree of heterosis might occasionally be dependent on such a small number of genes that approach to the F_1 type could be obtained in a homozygous strain. Success in such a system as RICHEY has proposed in his Convergent System of Improvement in Maize is, for this reason, not wholly idealistic. I have had at least two populations in tobacco where the theoretical expectancy appeared to have been realized; and MÜNTZING (1931) had one in a cross between *Galeopsis Tetrahit*×*G. bifida*. I do not agree with MÜNTZING, however, that such cases can be relied upon as proof of the linked gene theory of heterosis.

On the other hand, a substantial amount of critical evidence in favor of the theory is to be found among polyploid plants. To weigh this evidence properly, it is necessary to discuss briefly the general effects of polyploidy.

It is ordinarily supposed that any increase in the number of complete genoms is accompanied by increase in size and vigor of the organism. But is this supposition sound? I believe that it is unsound. Some dozen or so haploids have been described in genetic literature. Probably hundreds could have been described had it been thought desirable. At this laboratory alone, at least ten have been studied. They are invariably smaller and less vigorous than diploids. Thus there does seem to be some reason why the common somatic condition, persistent so long under natural selection, is a better running physiological machine than the haploid condition. But it does not follow that higher polyploids are still larger and more vigorous than diploids.

The idea that doubling the chromosomes of a species produces a giant form arose from the use of the term *gigas* to describe the first autotetraploid of *Oenothera Lamarckiana*. The name clung when similar traits were frequently found to characterize the autotetraploids of Datura (BLAKESLEE, BELLING, and FARNHAM 1923), Solanum (JORGENSEN 1928, LINDSTROM and KOOS 1931), and of other species. But the plants are not giant forms. Their distinguishing features are stouter and stockier stems, somewhat larger leaves and flowers, and, in particular, very rugose leaves. And these characters are by no means designating traits for all autotetraploids or higher autopolyploids. So far as I am aware, few monocotyledonous

autopolyploids are distinguishable from the diploids from which they arose. As evidence on this point there are the tetraploid maize plants produced through heat treatment by RANDOLPH (unpublished), the numerous tetraploid Tradescantia species (ANDERSON and DIEHL 1932, ANDERSON and WOODSON 1935), a tetraploid Rhoeo (SAX, unpublished), several Iris species (ANDERSON, unpublished; these are difficult to distinguish from diploids), and several types of Phleum (GREGOR and SANSOME 1930). In fact, the only giant forms that appear to be autotetraploids are *Iris mesopotamica*, a bearded iris, and *Iris Sintenisii*, a beardless iris (SIMONET 1934). In the dicotyledons, autopolyploids are not always distinguishable. BLACKBURN (1927, 1928) has reported them in Silene. I have seen them in Petunia and Lycopersicum (distinguishable with difficulty). And apparently, from the descriptions, it is not uncommon to find difficultly distinguishable tetraploids in Datura, Solanum, and other members of the Solanaceae. I suggest that *gigas* forms are special cases found only in certain groups, probably where the epidermis is soft. It seems likely that more indistinguishable autotetraploids will be found when more chromosome determinations are made at random, as in ANDERSON'S work. Hitherto, *gigas* forms have had more attention from cytogeneticists than ordinary types.

This matter has been stressed because it gives one a clearer idea as to how much, or how little, vigor should be allowed for when comparing autotetraploids and allotetraploids. These terms are convenient, but they are not alternative descriptions. On the contrary, they form a continuous series. The best cytological criterion for separating them is whether the chromosomes form bivalents (auto), or trivalents and quadrivalents (allo) at meiosis. Now, most polyploid series of natural species are assumed to have been derived from hybrids by chromosome increase; yet there is scarcely any evidence that the vigor of members of a polyploid series rises with increase in chromosome number. Only in Erophila has it been maintained that length of leaf increases with chromosome number (WINGE 1933). If this is not the rule, then either amphidiploids show no hybrid vigor, or the natural polyploid series do not originate from hybrids. We shall show that many artificially produced amphidiploids do exhibit hybrid vigor comparable to their diploid parents, thus indicating some validity to the linked gene hypothesis and also indicating that hybridization has less to do with natural polyploidy than is generally assumed.

It would hardly be defensible to try to diminish the value of hybridization too vociferously. HUSKINS (1930) has shown that there is high probability that *Spartina Townsendii* is an amphidiploid arising from a cross between *S. alternifolia* and *S. stricta;* and it is so vigorous a type that it has displaced the parental forms where in contact, spread all over southern

England, and passed over to France, since 1870. But if one thinks a moment, it is clear that hybridization followed by amphidiploidy has been overemphasized as an evolutionary factor. Species hybridization under natural conditions is very rare. Amphidiploidy is rare. The second event must wait on the first. Thus it seems that the resultant new species must have to have extraordinarily favorable characteristics and must meet extremely favorable environmental conditions in order to persist. On the other hand, autopolyploidy is extremely common. It can be produced at will, at least in certain groups, by temperature extremes and by mutilation. All that is needed for persistence under competition is a slight selective advantage in form and function and genetic changes (known to occur) bringing about normal pairing at meiosis. In fact, chiasma frequency in most plants is so low that polyvalent association is not a material obstacle. We know little about the actualities of such a scheme. But ANDERSON'S and WOODSON'S survey (1935) of the Tradescantias in the United States, probably the most careful and extensive investigation of its type, is very suggestive. Tetraploid forms appear to be increasing more rapidly than diploid forms around the periphery of the distribution of several species.

About forty amphidiploids arising from hybrid parentage are known. From a careful study of the somewhat fragmentary breeding records, I believe it is clear that, unless additional variables are present, these amphidiploids possess the characteristics of the diploids and breed true to them. This evidence definitely proves the hereditary basis of heterosis and is consistent with the linked gene theory. It fails to give us a clear picture of the activity of the genes concerned; but shows that the problem of heterosis is the problem of the inheritance of quantitative characters.

Any analysis of the amphidiploid evidence in relation to heterosis is rational and consistent only if the action of certain extraneous variables is kept in mind. The more important of these complications are as follows. First, it must be remembered that, though normally heterosis increases roughly with genetic dissimilarity within a genus, different genera can not be compared easily because each genus has its own scale of manifestation. Again, it often happens that when rather distantly related species are crossed, there is a disharmonious relation between the components that results in dwarf forms; and these dwarf forms frequently show their nature by monstrous developments in particular organs. Second, one must not forget that the amphidiploids used in genetic research are types that have not been made into smoothly running physiological entities by long periods of selection. Numerous cytological aberrations appear—aneuploidy translocation, inversion, deletion, and the like—in addition to other disturbing internal factors, like secondary association, and to disturbing external factors. The frequency and type of the cytological disturbances

appear to show relationship (1) with mode of origin of the hybrid, whether by somatic doubling or by polyploid gametic unions, and (2) with mode of origin of the components, whether polyploid or not, in the genetic rather than the cytological sense. Little is known about the possibility of external disturbances, but ANDERSON'S investigations indicate that an autotetraploid may react to conditions in a somewhat different manner than the diploid from which it came. Third, any possible changes due to chromosome doubling itself must be discounted.

The only amphidiploid known to have arisen by somatic doubling is *Primula kewensis*, which came from amphidiploid buds on a chance cross between *P. floribunda* and *P. verticillata* (NEWTON and PELLEW 1929). As shown by artificial hybrids made by COUTTS, the hybrid exhibits definite heterosis, particularly in the leaves and flowers. In *P. kewensis*, these manifestations are slightly exaggerated, as if a little of the *gigas* condition had been added by the chromosomes' doubling. The species breeds true in the horticultural sense of the term, though not strictly true in the genetic sense. Of 287 plants raised at John Innes Horticultural Institution, 261 bred true, and 26 plainly showed variation from type. Several variants studied showed chromosomal aberrations.

The remaining amphidiploids, though some of them may have arisen by somatic doubling, appear to involve diploid gametic unions. They derive either from $2N \times 2N$, or from $(2N \times N) \times N$ by backcrossing a triploid. Some few were too sterile to afford any data on the matter in hand, as in Crepis (BABCOCK and NAVASHIN 1930); and some have not been described sufficiently for a satisfactory judgment, as the Pimpinellifolia-villosa hexaploid rose (BLACKBURN and HARRISON 1924); but most of them afford useful information on the point under consideration. The greatest amount of work has been done on the wheat relatives, the tobaccos, and the cabbage-radish hybrids. The other examples are more scattered.

The amphidiploids among the wheat relatives ordinarily show a considerable degree of segregation due to chromosome aberrations or to polyvalent chromosome unions, as is to be expected where at least one of the parents is nearly always a natural polyploid. Moreover, the components of these hybrids are often too different genetically to show heterosis because of physiological disharmony. But apart from the interference of chromosomal aberrations, the double diploids breed true to the characters of the diploid hybrids, vigor included. The constant wheat-rye hybrids of Rimpau (LINDSCHAU and OEHLER 1935) are intermediates without noticeable heterosis; the constant wheat-rye hybrids of MEISTER (1928, LEVITSKY and BENETZKAIA 1931, LEBEDEFF 1934) show marked vigor in many of the combinations made. The $(7 \times 14) \times 2$ chromosome hybrids are not infrequently weak types (KIHARA and KATAYAMA 1931, TAYLOR and

LEIGHTY 1931, OEHLER 1934); but some of them are quite vigorous (PERCIVAL 1930, TSCHERMAK and BLEIER 1926, TSCHERMAK 1929, 1929a, 1930, 1934).

Within the cabbage tribe, two notable amphidiploids have been produced. FRANDSEN and WINGE (1931) made crosses between the turnip and the swede (10+18), which manifested marked heterosis; but the fertile amphidiploid which appeared was still more vigorous, and its progeny apparently retained this vigor. The new species, *Brassica napocampestris*, was intermediate between the parental species in its general traits. There was also considerable heterosis in the true breeding balanced forms of Brassicoraphanus originated by KARPETSCHENKO (1927, 1927a, 1928. 1929) from crosses between *Raphanus sativus* (9)×*Brassica oleracea* (9). The unbalanced forms usually showed their condition by aberration, including dwarfing. Another constant Brassicoraphanus amphidiploid has been isolated by TERASAWA (1932). *B. chinensis* (n - 10) was the cabbage used. Nothing is said about heterosis. It is unfortunate that the varieties in the crosses of KARPETSCHENKO—and apparently in those of TERASAWA —did not produce hybrids of such formidable vigor as those obtained by GRAVETT (1914) and SAX (unpublished).

A number of amphidiploids have been obtained from Nicotiana hybrids. In most cases one component was already a polyploid, *N. Tabacum*, *N. rustica*, and *N. Bigelovii*. This introduced some cytological irregularity. Some, moreover, were combinations which are ordinarily rather weak—*N. glutinosa*×*N. Tabacum*, and *N. suaveolens*×*N. Bigelovii*. At least one, *N. Tabacum*×*N. glauca* (TERNOVSKY 1935), showed so much unbalance that a constant race was not obtained. But, in general, the amphidiploids bred true to the characteristics of the hybrid; and where heterosis was present, it was continued. *N. glutinosa*×*N. Tabacum* was, in CLAUSEN and GOODSPEED'S case (1925), not markedly vigorous, and in my experience the hybrid has been dwarfish; but in TERNOVSKY'S case it exhibited "völlige Lebensfähigkeit und eine energische kräftige Entwickelung."

The amphidiploid of *N. Tabacum*×*N. rustica* (EGHIS 1927, RYBIN 1927), which came from a triploid backcrossed to *N. rustica*, was exceptional in that it showed less heterosis than the hybrid diploids. Nevertheless, it was notably vigorous and bred fairly true to its characteristics. Here the new species was really an octoploid derived from two tetraploid components.

The fertile double diploid hybrid between *N. Tabacum* and *N. sylvestris* (EGHIS 1930, RYBIN 1929) is again a special case in that *N. sylvestris* is a component of *N. Tabacum*. The new form showed heterosis; but I judge that the particular combination of varieties made gave somewhat less vigorous hybrids than are usually obtained.

The amphidiploids from *N. rustica*×*N. paniculata* (LAMMERTS 1931,

SINGLETON 1932) are again an instance of adding similar genes, for *N. paniculata* is a component of *N. rustica*. The hybrids are always intermediate in size, but show vigor in rapidity of growth and early maturity. LAMMERTS obtained both dwarf and giant races. SINGLETON obtained plants much like those of the F_1 generation.

The amphidiploid between *N. glutinosa* and *N. tomentosa* (GOODSPEED 1933, ELVERS 1934) had great vigor, even more than the diploid. The *N. suaveolens*×*N. Bigelovii* amphidiploid has not been described (GOODSPEED 1933).

The constant amphidiploid arising from a hybrid between *Digitalis ambigua* and *D. purpurea* (n = ca 112) described by BUXTON and NEWTON (1928) had a notable degree of heterosis in the leaves and apparently in the flowers.

CRANE and DARLINGTON (1927) indicate that the Loganberry, presumably an amphidiploid of unknown parentage, exhibits a high degree of vigor; and my own observations corroborate this view. Its progeny are somewhat variable, as is to be expected from the cytological complexity of Rubus.

HIORTH (1934) describes a polyploid hybrid between *Collinsia bicolor* and *C. bartsiaefolia* which has been constant for five generations. Heterosis is apparently marked by "thick leaves, broad with rounded tip," and large flowers. HUSKINS' (1930) description of the Spartina case has already been mentioned. SKOVSTED (1929) describes a constant amphidiploid showing marked heterosis arising from a cross between *Aesculus pavia* (20 small chromosomes) and *A. Hipposcastaneum* (20 large chromosomes). WINGE (1933) describes a constant amphidiploid arising from two types of *Erophila verna*, where n equals 15 and 32 respectively. There is a fair degree of variability in this form; but the amphidiploid is much more robust than either of the parents, at least in the rosette stage. SKALINSKA (1935) describes a peculiar amphidiploid coming from *Aquilegia chrysantha* (n = 7) and *A. flabellata nana* (n = 7). The plants of the first species are 70–80 cm in height, while those of the second species are only 30–40 cm in height. The F_1 individuals are much larger than either parent, ranging from 100 cm to 110 cm. The tetraploids are only about 70 cm in height. They show marked heterosis in size of rosette and in number and stockiness of the stems produced, but they certainly are less vigorous than the diploids. The variability of the forms indicates additional variables at work.

Finally, MÜNTZING'S (1931, 1932) admirable work in synthesizing *Galeopsis Tetrahit* from *G. pubescens* crossed with *G. speciosa* should be emphasized. It is the most complete description of any amphidiploid. The plants, except for two dwarf segregates, were generally larger and more vigorous than the parental species.

On the other hand, certain crosses exhibit no noticeable heterosis either as diploids or amphidiploids. This is apparently the case in MÜNTZING'S (1935) example in Phleum and in NILSSON'S (1935) example in Festuca. Such instances are to be expected.

THE DEPENDENCE OF HETEROSIS UPON GENETIC DISPARITY IN THE PARENTAL STOCKS

In 1912 EAST and HAYES expressed the opinion, based on much too meagre evidence, that the amount of heterosis expressed in an F_1 plant is roughly proportional to the genic differentiation in the two parental stocks, provided normal ontogeny is possible. Since that date, I have had the opportunity of studying a variety of crosses in the following genera: Antirrhinum, Aquilegia, Begonia, Beta, Capsicum, Campanula, Catalpa, Chelidonium, Crepis, Cucumis, Cucurbita, Datura, Delphinium, Digitalis, Eschscholtzia, Euchlaena, Fragaria, Glycine, Gloxinia, Impatiens, Linaria, Linum, Lycopersicum, Lythrum, three genera of the Malvaceae (Althaea, Lavatera, and Malva), Nicotiana, Oxalis, Pelargonium, Petunia, Phaseolus, Pisum, Primula, Salpiglossis, Solanum, and Zea. In addition, about a score of different natural hybrids have been examined, these being mostly arboreal types. And the bearing of the data collected on the problems of heterosis has never been forgotten.

The conclusion given in the preceding paragraph has been confirmed. Heterosis does increase as the genetic disparity of the parental stocks increases, on the average. Crosses between pedigreed inbred stocks exhibit diminishing heterosis as genetic relationship increases. Unrelated autogamous varieties show more heterosis when crossed than do related varieties. Crosses in heterogamous stocks show less heterosis than crosses in autogamous stocks. Increase in heterosis is noticed when heterogamous stocks have been successively selfed before crossing. This is particularly apparent in self-sterile species (certain Nicotianas, Lythrum, Oxalis) that can be inbred by special technique. Interspecific crosses show more heterosis than intraspecific crosses. This is true, however, only when the genic differences "nick," as they say in stock-breeding, and there is no interference with developmental processes; but if the genic differences fail to "nick," and there is disharmony, then dwarfs are obtained. In such cases, heterosis may be very marked in a whole series of interspecific crosses; yet other apparently similar combinations give dwarfs; and there are no intermediates between the two types. Of course, one can not say with certainty that the dwarf hybrids would have been vigorous hybrids had it not been for the failure of the genic combinations to work together harmoniously, since dwarfing may come about for other reasons (single genes are known to produce it); but this is the indication. In Nicotiana and Fragaria, two

genera with which I have worked intensively, hybrids between species belonging to different subgenera show more heterosis than hybrids between species belonging to the same subgenera; but the former also show more dwarf combinations than the latter. The end of the series, theoretically, should be intergeneric hybrids, since interfamilial hybrids have not been obtained; and there is some evidence that this is so. It is difficult to obtain intergeneric hybrids that are not abnormal; yet the greatest amount of heterosis ever noted is the Raphanus-Brassica combination.

On the other hand, our more recent experiences indicate that the earlier conclusion must be somewhat modified. Particular genes, or perhaps particular combinations of a small number of genes, have special effects. Different maize varieties, and different tobacco varieties, that are not particularly distinct genetically, as is shown by the behavior of a series of crosses among them, show markedly different degrees of heterosis when crossed with more diverse varieties or species. This experience appears to indicate that certain genes can exert greater effects than other genes, and also that a given gene can have greater effects in some combinations than in others.

Again, the effects of heterosis can not be compared in different genera. Each genus must be considered by itself. In other words, the genetic evidence indicates that certain generic groups are varying more than others, and in ways peculiar to the group concerned. This is not to say merely that one genus may show greater diversity than another, or even that one genus may show greater speciation than another. These are truisms; though it should not be assumed that speciation is a perfect measure of genetic diversity. What I really have in mind is that present-day genetic variability (that is, current or recent mutation rates) is very low in some groups and very high in others.

THE CHARACTER OF THE MANIFESTATIONS OF HETEROSIS

A second point, not so obvious as it may seem, is that heterosis is most aptly described by the old term *hybrid vigor*. In considering the various hybrids with which I have had to deal, a special effort has been made to determine just what characters are affected. Roots, stems, hairs, leaves, flowers, fruit, and seeds have been studied with care. As a result, it can be said that hybrid vigor is something which almost invariably concerns the plant—or the animal—as a whole. Its effect is comparable to the effect on a plant of the addition of a balanced fertilizer to the soil, or to feeding a more adequate and more chemically complete diet to the animal.

In plants the root system is increased, the branching is more profuse, the leaves are larger and more abundant; growth takes place faster—at least in the early stages— and often retains its pace longer before showing the characteristic sigmoid curve that indicates approaching maturity. I have

not studied the anatomical changes; but according to my colleague, Professor I. W. BAILEY, the wood of the very vigorous hybrid poplars (SCHREINER and STOUT 1934, SCHREINER 1935), when compared to that of their parents, shows just those changes that occur when tree species are grown under "good" rather than under "poor" conditions. Generally speaking, cell division is more noticeably influenced than cell size.

Hybrid vigor may be shown by an early maturity or by a late maturity. It all depends on the type of the genetic union. It is exhibited early. It is expecially marked in seedlings and the rosettes of species that form rosettes; but it is also apparent at late periods in the vegetative part of the life cycle. It is not marked in fruits, however, and is seldom noticeable in flowers I take it that this is partly because the general vegetative impetus has, in a sense, lost its force by the time the plant has reached sexual maturity. It also appears that the phenomena attending the reproductive process stand somewhat apart from ordinary vegetative growth. At the same time, it should be noted that *preparation* for reproduction is vegetative; hence, heterosis is frequently shown in the *profusion* of flowers and fruit.

In this connection I have studied the inheritance of a large number of characters which might be expected to show heterosis from *a priori* reasoning, but which do not show it. They are characters determined by a small number of genes and affecting particular organ systems. They do not, as far as can be judged, affect the general efficacy of the whole physiological machine. I will mention two examples,—leaf number on the main stem of the tobacco plant, and number of rows in the ear of maize. Varieties may sometimes be crossed which differ in the genes which determine these characters, as *AAbbCCdd* and *aaBBccDD*, thus giving a greater number of leaves, or of rows per ear, in the F_1 individuals than either parent possesed. But the segregation of the determinants is relatively simple. In no case have I found the great genetic complexity involved that is so obvious in heterosis, nor can the effect be described as one of increased general metabolic efficiency.

It is evident, then, that heterosis is a resultant exhibited by the organism as an entity and characterized, as CHILD might say, by a change in the metabolic gradient. This is what is shown so nicely by ASHBY's logarithmic curves. Heterosis is ordinarily manifested by a rise in the "efficiency index," in the sense with which ASHBY used the term. And the unity of its expression might well lead a physiologist to think of it as a "unit character" with the notion which that expression conveyed in the early days of genetics.

These facts clearly teach that, in any given population, more *different* allelomorphs are present among the genes influencing the physiological efficiency than among the genes influencing the morphological pattern. Just why this should be the situation is less obvious. Naturally, the familial

generic, and specific characteristics of a genetic group are retained over long periods; hence, one may assume that the more conservative organ systems, presumably the older systems phylogenetically, are relatively less affected than others by the common run of gene mutations. It is also probable that the genes themselves vary in stability. There is evidence in favor of both propositions. I suggest, therefore, that the clearest and most reasonable interpretation of the facts lies in a combination of these two viewpoints based on the idea that gene mutation rates vary with function rather than with specific identity.

Basic changes in morphology, meristic variation, and even changes in certain ratios of parts (that is, shapes) occur relatively rarely; and when they do occur, the differences between the mutants and the wild types are found to depend upon a small number of genic changes. The changes involved in heterosis, though they are often insignificant phenotypically, are nearly always found to depend upon a large number of gene mutations; and they seem to be superimposed upon the first type. The first type appears to set the character of the reaction, while the second type sets the speed of the reaction. Heterosis, then, is largely concerned with changes in the speed of the various physiological reactions. And the genes controlling the speed of ontogenetic processes have higher mutation rates than the genes controlling the nature of these processes.

THE NATURE OF HETEROSIS

If heterosis is a phenomenon produced when numerous linked "dominant" genes are opposed to homologous "recessive" genes, and if these genes are mainly though not wholly those which influence general vigor, any further insight into the problem can come only from a clearer view of the manner in which these genes behave during development. We may assume that all genes are distributed by the same mechanism, but we must inquire as to whether all genes function similarly during ontogeny. The evidence indicates that genes may be divided into two main groups functioning differently, and that their behavior furnishes us with the solution of the problem. The first group causes breakdowns in physiological processes; the second does not. Naturally, the distinction between the two classes is not always clear cut. But most of the mutations used in linkage experiments may be placed in the first category without serious error. For example, in Drosophila, the lethals, the eye colors, the body colors, the bristle deficiencies, the wing simplifications, and all similar genic effects are to be included. In plants, chlorophyll deficiencies, deficiencies producing dwarfness of the whole or of a part, sexual deficiencies, organ simplifications, and most color changes are to be admitted, though the latter are ordinarily defective only for non-essential developmental processes. These mutations

all tend to be recessive to the "normal" or "wild" type, and it is not hard hard to see why. Most organisms are 2x-cylindered engines, so to speak, but can run on x-cylindered engines. Any defectiveness in one of the paired genes is compensated by normality in the other, and the processes in which these genes are involved go on as usual. The only easily imagined way in which a defect may appear to be dominant is when a defective gene impairs cytoplasmic properties to such an extent that there is a reflected effect on the activity of the normal gene; and such mutations would ordinarily be weeded out rapidly.

Genes of this defective type are the masked deleterious genes that are uncovered by inbreeding. In fact, inbreeding is often described as a process of purification, whereby a strain is purged of its pernicious elements. But I have been driven to conclude that the elimination of deleterious recessives is of little importance in practical breeding and of no consequence whatever in the solution of the problem being considered here.

A huge number of maize stocks have now been inbred through self-fertilization for periods of from ten to thirty years. The invariable experience is this: There is rapid decline in vigor during the early years, becoming less marked until, after approximately eight years, there is no further detectable decline. There is a similar unmasking and elimination of deleterious recessives which gradually diminishes and disappears; and there is segregation into differently characterized biotypes. ***But these purified biotypes exhibit as great or greater manifestations of heterosis when combined after they no longer segregate defective recessives as they did earlier.*** It follows that heterosis is not concerned with defective genes but rather with the different genic isomers of the physiologically active and more or less normal genes belonging to our second category. It also follows, since heterosis is almost omnipresent in crosses between inbred lines which have had virtually all defective genes eliminated, that non-defective genic isomers greatly outnumber the defective ones.

These conclusions are fairly obvious when one considers the matter. If *A* and *B* are normal genes and *a* and *b* are defectives, it is usually found that *Aa* or *Bb* individuals show a close approach to complete dominance. In other words, *Aa* and *Bb* individuals can not be separated from *AA* and *BB* individuals. How, then, are our so-called "dominants" and recessives to be opposed to each other by crossing, since we do not use *AAbb* and *aaBB* individuals as our pure strain components? No! Heterosis must be interpreted on the basis of the behavior of non-defective allelomorphs. The decline in vigor through successive self-fertilizations must be due to increase in homozygosis of genes belonging to this category. And JONES' hypothesis is a linkage hypothesis rather than a "dominance" hypothesis,

unless "dominance" and "recessiveness" in the classical sense are attributes of non-defective gene behavior.

Since one may say, in short, that the final analysis of the heterosis problem depends on the type of behavior exhibited by the genes affecting quantitative relationships, the question is mainly as to how this behavior is to be described. The literature on the subject is too voluminous to consider in detail here. I can only say that the universal experience is to find "dominance," in the classical sense, virtually absent. I have, in my own papers, presented analyses of numerous studies during a period of 27 years; and I will call attention only to an investigation of species hybrids (EAST 1935), where the behavior of the genes involved indicated that each is an active pattern former. This particular study could not be carried beyond the F_1 generation; but numerous other investigations of frequency distributions from intraspecific crosses show that a simple additive interaction of multiple factors is inconceivable (the first suggestion of this was probably in EAST 1913). RASMUSSON (1933), therefore, has proposed a gene interaction hypothesis to account for the facts actually observed. He assumes that "the effect of each factor on the genotype is dependent upon all the other factors present, the visible effect of a certain factor being smaller the greater the number of factors acting in the same direction." Thus A and B, acting alone, may each have an effect equal to 1; but $A+B$ have an effect less than 2. Now, it is unnecessary to accept RASMUSSON's hypothesis precisely as he has presented it. It may need some modification.[1] But it is safe to say that all plant breeders agree that a non-arithmetical accumulative hypothesis is required. On this basis I wish to make a suggestion that enables us to visualize the heterosis situation with considerable clarity. The suggestion is simply this: *The cumulative action of the non-defective allelomorphs of a given gene approaches the strictly additive as they diverge from each other in function.* It is impossible, at the present time, to give rigorous proof of this theorem. All I can say is that a long experience with this type of gene leads me to believe that it is a close approximation of the truth.

[1] Rasmusson was impressed, as all plant breeders have been, by the fact that many more different genes are present in the average population of plants than is usually assumed. This point is undeniable. Similarly, it can not be denied that most observations on quantitative gene action are uninterpretable on the basis of simple arithmetical increments. Such interpretations have been used, but only as diagrammatic representations. I have used them myself in several early papers, but I was careful to point out, in various places, that this was a simplified representation used only for ease of presentation. There may be gene action at times similar to that suggested by Rasmusson. There is also gene action where the presence of a basic gene is necessary for the expression of modifiers. But in growth phenomena, one is dealing with ratios, not simple additions. It is likely, therefore, that many first approximations, in dealing with quantitative inheritance, must be interpreted on a simple logarithmic basis or by means of higher geometric series. It is not improbable, however, that series of genes show diminishing returns over what they would otherwise be expected to produce in a logarithmic or other geometric basis.

The two types of genes may be assumed to behave as follows. If A_1 is a normal gene and a differs from it only by some degree of defectiveness in the process that A has been capable of performing, then the capacity of a for performing the function of A_1 approaches zero. Yet A_1 in combination with a performs its full function, and A_1a and A_1A_1 individuals are indistinguishable. If, on the other hand, A_1 takes on a series of isomeric conditions $A_2 \ldots A_4$, each having positive active functions diverging further and further from those of A_1, then the combination A_1A_4 may be supposed to have greater physiological efficiency, provided the various functions are harmonious, than A_1A_2. Quantitatively the action of A_1a is virtually A_1+0; while the action of A_1A_1 is not $2A_1$ but $2A_1-\alpha$ and α is virtually equivalent to A. Similarly, the action of A_1A_2 is $A_1+A_2-\beta$, that of A_1A_3 is $A_1+A_3-\gamma$, and that of A_1A_4 is $A_1+A_4-\delta$; and $\alpha>\beta>\gamma>\delta$.

Actually, many critical data are available only on comparisons between A_1+defective a and A_1+the identical allelomorph A_1. But since the frequency distributions where genes at different loci accumulate quantitative effects sometimes demand a diminishing returns interpretation, it is plausible to postulate a similar activity for non-defective allelomorphs at a single locus. Moreover, I have had several populations in my studies of size inheritance where very few gene differences at separate loci seemed to be involved; and the actual segregations obtained appeared to be more easily explained if segregation of pattern effects such as $A_1A_4B_1B_4$ (though probably not so simple) were assumed. There is marked positive skewness to the distributions; yet it appears to be impossible to fix the characteristics of the extreme positive variants, and the difficulty of fixing any positive variants is much greater than that of fixing minus variants. This last statement may seem to contradict an earlier one to the effect that heterosis possibly is fixable in diploids in rare cases. I believe that the two cases are not wholly incompatible but depend upon the type of the allelomorphs involved.

SUMMARY

1. Various lines of evidence are cited which show that ASHBY'S physiological theory of heterosis is unsound in all its essential features.

2. The experimental evidence on amphidiploids is examined for its bearing on heterosis. The data show that heterosis is gene-controlled, since amphidiploids breed true (with certain explainable exceptions) to the vigorous conditions exhibited by the original hybrids. The facts are compatible with a theory of linked gene inheritance; and this is considered to be the fundamental basis of JONES' theory, rather than the dominance idea by which it is usually identified.

3. Reasons are given for thinking that autopolyploidy has been a more

important factor and allopolyploidy a less important factor than is generally assumed in the origin of natural polyploid series.

4. *Gigas* types are found not to characterize all polyploids. They are rare in monocotyledonous species. It is suggested that they occur most frequently when the epidermal tissues are soft.

5. Experimental studies on hybrids in 37 genera, in addition to examination of various natural hybrids, have confirmed an earlier conclusion that heterosis increases with genetic disparity between the parents. It is found that manifestations of heterosis are different in type and extent in different genera, indicating that current mutation rates in certain groups are much greater than in others.

6. Observations on the characters affected under manifestations of hybrid vigor show that heterosis concerns the plant as a whole. ASHBY'S "efficiency index" leads to the same conclusion. It follows that physiological efficiency is governed by numerous genes that have mutated frequently. Structural variations, on the other hand, are less common, though more noticeable, and involve fewer gene differences. It is suggested that structural variations and physiological efficiency variations belong to different categories. The first type is concerned with reaction character, the second with reaction speed. Heterosis is largely a matter of reaction speed.

7. Inbreeding commonly results in a diminution in vigor and an unmasking of deleterious recessives. These recessives are mostly defective gene mutations. Crosses between the "purged" inbred strains manifest a high degree of heterosis if the parental stocks are not too closely related. It follows that the presence of defective genes hidden by normal dominants has little to do with heterosis. It also follows that normal, positive, active genic allelomorphs are much more common than defective genic allelomorphs.

8. A consideration of the known behavior of normal and of defective allelomorphs, such as A and a, also shows that such combinations can not be effective in producing heterosis, for A is usually completely dominant to a. The effect of AA is not substantially greater, therefore, than that of Aa. Thus heterosis must be interpreted through the behavior of "normal" allelomorphic series. The key to heterosis is the inheritance of quantitative characters.

9. Since all experiments on quantitative characters have shown that "dominance," in the classical sense, is virtually absent, and that each gene of a bivalent combination actively affects the end result, a theory is suggested for explaining heterosis which combines the essential features of JONES' hypothesis, of RASMUSSON'S hypothesis concerning the behavior of genes influencing quantitative characters, and of the earlier idea that heterozygosis *per se* is effective.

If numerous non-defective allelomorphs are common in any given species heterosis can be accounted for as follows. The effect of A_1A_1 is not $2A_1$ but $2A_1-\alpha$ where the value of α approaches the value of A_1. But if $A_1 \ldots A_4$ is a series of non-defective allelomorphs of A_1, where their functions depart from those of A_1 on an ascending scale, then the resultant effects may be visualized as A_1A_2 equaling $A_1+A_2-\beta$, A_1A_3 equaling $A_1+A_3-\gamma$, and A_1A_4 equaling $A+A-\delta$, where $\alpha>\beta>\gamma>\delta$.

This theory appears to be compatible with the known facts, especially those accumulated in experiments on the inheritance of quantitative characters.

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THE NATURE OF THE INTERACTION OF GENES AFFECTING FOUR QUANTITATIVE CHARACTERS IN A CROSS BETWEEN HORDEUM DEFICIENS AND HORDEUM VULGARE

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INTRODUCTION

QUANTITATIVE characters are of extreme importance in economic plants. Because of difficulties involved in studying the inheritance of these characters less is known concerning their genetics than is the case for the qualitative characters.

SAX (1923), GRIFFEE (1925) and SIRKS (1925) were the first to study factors for quantitative characters by means of their association with factors for the production of qualitative characters. This method was used later by IMMER (1927). LINDSTROM (1926, 1928, 1929, 1931), who has studied the linkage between genes for quantitative and those for qualitative characters in the tomato, was the first to point out the possibilities of studying the inheritance of genes differentiating quantitative characters by means of their linkage with genes differentiating qualitative characters. LINDSTROM'S studies furnish rather conclusive evidence that major factors for size of fruit occur in the tomato. HAYES and HARLAN (1920) showed that there were at least three factor pairs affecting internode length in the spikes of barley. They found that these genes had different effects and were cumulative. Some long and short internode types may be differentiated by one factor pair and others by two or three. WEXELSEN (1934) studying genes influencing internode length of the barley spike substantiated these results and pointed out that the genes are not necessarily alike in their dominance relationships, since L_1, L_2 and L_3 were found to be intermediate in a heterozygous condition, L_4 to be nearer to the short type, and L_5 nearer to the long type. RASMUSSON (1935) in a study of quantitative characters in Pisum demonstrated that two main factors, both showing partial dominance towards lateness of maturity, were at work in the material investigated. He estimated that they were responsible for about half the genic variation within the F_2 populations, whereas the other half was believed to be due to modifiers. He found that these genes were not strictly additive in their combined effect and this supported his interaction hypothesis.

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This article reports a study of the effect of genes associated with color of glumes, type of spike and habit of growth upon yield of seed per plant, number of spikes per plant, height of plant, and length of awn. These include genes differentiating the qualitative characters in question as well as those which may be linked with them and which affect the quantitative characters being studied. It is apparent then that in part at least the effect of the differential genes in certain chromosomes is being measured, and it should be possible to establish the nature of the interactions of the genes located in different chromosomes.

MATERIALS

The material consists of data from F_1, F_2 and F_3 generations of a cross of B 1 and Brachytic. The contrasted characters of the parents are as follows:

Character	*B 1*	*Brachytic*
Color of Glumes	Black	White
Type of spike	deficiens	vulgare
Type of growth	normal	brachytic
Yield of seed per plant in grams	$1.9 \pm .07$	$4.0 \pm .25$
Number of spikes per plant	$3.8 \pm .11$	$4.7 \pm .25$
Height of plant in inches	$25.6 \pm .24$	$16.1 \pm .15$
Length of awn in mm	141.6 ± 1.19	$71.0 \pm .71$

B 1 is a standard stock used in linkage studies at the University of Minnesota (DAANE 1931) and has been carried from individual plant selections in the crossing plots. The parent material used as checks in this study was from the progeny of plants used in making the cross. The crosses were made by Dr. F. J. STEVENSON. The Brachytic parent was obtained from Dr. L. J. STADLER, University of Missouri, and was selected by him from a population of the variety Himalaya, the seed of which originally came from the Montana Experiment Station.

METHODS

Planting, harvesting and measuring. The F_1 and F_2 were grown in the summer of 1932 and the F_3 was grown in 1934 in 6 foot rows and spaced 3 inches apart within the row. The B 1 and Brachytic parents were grown as a check in rows adjacent to the F_1. The progeny from nine plants of the B 1 parent were grown with the 20 F_2 families, the nine B 1 families being distributed at random throughout the 20 F_2 families. As a further check the progeny from one plant of the Brachytic parent were grown at the end of the series of plantings adjacent to the progeny from one plant of the B 1 parent. Thus since the F_1 was grown some distance from the F_2 plants any comparison of the two will have to be made through the par-

ents. At the time of harvest each plant was pulled, its height measured in inches, and the spikes cut off and placed in an envelope. During the following winter the plants were classified; and the number of spikes per plant, the length of awns in millimeters, and the weight of seed in grams were recorded. In the F_3, 32 seeds of each F_2 plant were sown and later notes on the mature plants were taken in the field. Each family was studied on the individual plant basis and classified as homozygous or segregating for each of the three qualitative characters involved.

Statistical methods. First, the data were separated into 27 different genotypes on the basis of the factor pairs differentiating the three qualitative characters. Then the sums of squares, variances and regression coefficients were obtained for each genotype. The variances used in calculating the t values were corrected on the basis of the regression coefficients, FISHER (1934, p. 257). The variances for weight of seed per plant were corrected on the basis of the regression coefficients of weight of seed on the number of spikes; those for number of spikes on the basis of the regression coefficients of number of spikes on weight of seed; those for height of plant on the basis of the regression coefficients of the height of plant on length of awn; and those for length of awn on the basis of the regression coefficients of length of awn on height of plant.

From the data analysed in this manner it was found that the variances from the homozygous black, the heterozygous black, and the homozygous white glume segregates did not differ materially. Therefore a generalized standard deviation would be applicable. Consequently the total variance for comparing plants of the genotypes *BB*, *Bb*, and *bb* was obtained by adding the sums of squares for each of the nine genotypes resulting from a segregation of the genes differentiating type of spike and habit of growth and dividing by the appropriate degrees of freedom. The method is illustrated in the following tabulation for weight of seed per plant:

GENOTYPE	N	D.F.	NUMBER OF SPIKES (x) $S(X^2)-N\bar{X}^2$	$S(XY)-N\bar{X}\bar{Y}$	WEIGHT OF SEED (y) $S(Y^2)-N\bar{Y}^2$	REGRESSION COEFFICIENTS Y on X	D.F.	REDUCED $S(Y^2)-N\bar{Y}^2$
vvBrBr	61	60	489.443	503.000	997.500	1.028	59	480.567
vvBrbr	130	129	846.531	1183.417	2488.324	1.398	128	833.954
vvbrbr	64	63	181.484	149.142	329.151	.822	62	206.587
VvBrBr	130	129	1578.992	1151.002	999.069	.729	128	160.050
VvBrbr	249	248	2375.406	1847.454	1864.521	.778	247	427.678
Vvbrbr	130	129	787.700	441.540	333.907	.561	128	86.405
VVBrBr	78	77	805.962	510.542	412.707	.633	76	89.300
VVBrbr	109	108	884.991	606.767	501.801	.686	107	85.789
VVbrbr	54	53	257.500	131.617	82.703	.511	52	15.430
Total							987	2385.760

The data are shown to 3 places to the right of the decimal point but in obtaining the constants used in this study the figures were carried to six places to the right of the decimal point. As can be seen from the tabulation this method of obtaining the variances took out the differences between genotypes and consequently the variance obtained was that within genotypes. For making comparisons between the nine genotypes resulting from segregation of the genes differentiating type of spike and habit of growth the variance was obtained for each by adjusting the sum of squares on the basis of their respective regression coefficients. The reduced sums of squares are shown in the last column of the above tabulation and of course the variance for each genotype is found by dividing the reduced sums of squares by the degrees of freedom left after subtracting from the original degrees of freedom the one accounted for by the regression coefficient. The t values to determine the significance of means and the t values to determine the significance of regression coefficients were obtained by use of methods given by FISHER, (1934) pp. 120 and 138 respectively. In determining whether differences between variances were statistically significant Fisher's formula (1934, p. 214) was employed. Differences giving odds as great as or greater than 19:1 against the deviations noted as being due to the errors of random sampling were considered as statistically significant.

That the regressions were obtained for each of the nine genotypes separately and adjustment of the variance for each genotype was made on the basis of its respective regression coefficient is important in that it shows what components of the variability are being controlled. By such a method the variability that is partially controlled is that due to environment and the genes independently inherited from those linked with and differentiating type of spike and habit of growth. For illustration let us consider the variances for weight of seeds per plant in which it was found that the general correlation coefficient between weight of seed and number of spikes within phenotypes was .81. This shows, as would be expected that the number of spikes per plant affects materially the yield of the plant. Now, since the purpose of this study is to measure differences between genotypes it would be desirable to have the number of spikes held constant within genotypes in so far as it affects yield. This would in no way interfere with the differences in number of spikes between genotypes, but would aid in the study of the genes differentiating the qualitative characters involved and any genes that might be linked with them. The completeness with which the effect of the linked genes is measured depends of course upon the closeness of the linkage relationship.

EXPERIMENTAL DATA

Inheritance of Color of Glumes, Type of Spikes and Habit of Growth, and their Linkage Relationships

A knowledge of the number of genes differentiating the qualitative characters and their linkage relationships is essential to a correct interpretation. The data bearing on this point are given in table 1. The proportion of homozygous black glume: heterozygous black glume: homozygous white glume segregates was 252: 504: 249; the proportion of homozygous deficiens: heterozygous deficiens: homozygous vulgare was 241: 509: 255; and the proportion of homozygous for normal habit of growth: those heterozygous for normal habit of growth: those homozygous for brachytic habit of growth was 269: 488: 248. These figures are based on the segrega-

TABLE 1

Linkage relationships between three qualitative characters as determined by partitioning χ^2 for goodness of fit into its components.

TYPE OF SPIKE vs. COLOR OF GLUMES		HABIT OF GROWTH vs. COLOR OF GLUMES		TYPE OF SPIKE vs. HABIT OF GROWTH		EXPECTED IF INDEPENDENT
GENOTYPE	OBTAINED	GENOTYPE	OBTAINED	GENOTYPE	OBTAINED	
VVBB	56	*BrBrBB*	70	*VVBrBr*	78	62.81
VVBb	115	*BrBrBb*	128	*VVBrbr*	109	125.62
VVbb	70	*BrBrbb*	71	*VVbrbr*	54	62.81
VvBB	134	*BrbrBB*	118	*VvBrBr*	130	125.63
VvBb	256	*BrbrBb*	257	*VvBrbr*	249	251.25
Vvbb	119	*Brbrbb*	113	*Vvbrbr*	130	125.63
vvBB	62	*brbrBB*	64	*vvBrBr*	61	62.81
vvBb	133	*brbrBb*	119	*vvBrbr*	130	125.63
vvbb	60	*brbrbb*	65	*vvbrbr*	64	62.81
P between .50 and .30		P between .80 and .70		P between .30 and .20		

tion as determined by classification of the F_2 and a progeny test in the F_3 generation. The expected ratio for all three characters based on the hypothesis that the contrasted characters are differentiated by 1 factor pair is 251.25: 502.50: 251.25. The P values obtained by applying the χ^2 test were found to lie between .98 and .95, .80 and .70 and .50 and .30 for color of glume, type of spike and habit of growth, respectively. It is apparent that the three qualitative characters are differentiated by a single factor pair.

The data concerning the linkage relationships of these three qualitative characters are given in table 1 also. The P values of χ^2 for independent inheritance for type of spike and color of glume, habit of growth and color of glume and type of spike and habit of growth lie between .50 and .30, .80 and .70, and .30 and .20 respectively. It may be concluded that the

genes differentiating the characters under consideration are located in different chromosomes. That color of glumes and type of spike are independently inherited has been shown by previous workers. For a review of literature on linkage relationships see DAANE (1931). It is apparent that any effect upon yield, number of spikes, height, or length of awn that may be found to be associated with any of these pairs of alleles will be independent, as far as linkage relationships are concerned, from the effect that any different pair of alleles might be exerting.

Genes Associated With Color of Glumes

The means of weight of seed, spikes per plant, height of plant, and length of awn for the three different genotypes are given in table 2. The genotype was determined by segregation in the F_2 and a progeny test in the F_3 generation; whereas, the measurements are for the F_2 generation.

TABLE 2

The means of four quantitative characters measured in the F_2 generation of a cross between B1, and Brachytic, classified into phenotypes and genotypes according to color of glumes.

PHENOTYPES	GENOTYPES	WEIGHT OF SEEDS IN GRAMS	NUMBER OF SPIKES PER PLANT	HEIGHT OF PLANT IN INCHES	LENGTH OF AWN IN MM
White	(*bb*)	4.7	5.9	24.4	117.3
Black	(*Bb*)	5.0	6.0	24.6	119.6
Black	(*BB*)	4.7	5.8	24.2	117.8

If a t value of 1.960 which gives a P value of .05 is taken as statistically significant, the seed yield of the *Bb* segregates was significantly greater than either those of the genotype *BB* or *bb;* whereas, the weights of seed per plant for the *BB* and *bb* segregates were practically the same. The t values were 2.501, 2.491 and .148 respectively. The only other t values exceeding 1.960 were those for *Bb* and *bb* in comparing length of awn and *Bb* and *BB* in comparing height of plant. However it can be seen from table 2 that in every case the mean of the heterozygote exceeds those of the two homozygotes for the four characters. Even though the differences between the two homozygotes and the heterozygote are small, there seems to be little reason for questioning their biological significance. Since the differences between the two homozygotes are neither consistent nor have t values sufficiently large to closely approach a P. of .05, it can be concluded that *bb* and *BB* do not have a differential effect upon weight of seed, spikes per plant, height of plant, nor length of awn.

These results can be explained by JONES' (1917) hypothesis to account for heterosis. If this explanation is accepted then the increases noted for the heterozygote must be due to favorable, and at least partially dominant genes, some of which must be located on the same chromosome with

B and others on the homologous chromosome with *b*. Furthermore, it must follow from the results obtained, if we are to accept JONES' hypothesis, that in the homozygous condition the genes located in the same chromosome with *B* have a similar effect upon the characters under consideration as do the genes linked with *b* when they are in a homozygous condition.

Genes Associated with Type of Spike and Habits of Growth

The data showing the reaction of the genes upon the four quantitative characters are given in tables 3 and 4. Table 3 is designed to facilitate an analysis of the differences between the *vv* and *Vv* segregates, the *vv* and *VV* segregates, and the *Vv* and *VV* segregates. These differences are shown for plants of the genotypes *BrBr*, *Brbr*, and *brbr*, making a total of nine comparisons for each of the four quantitative characters. Table 4 is the same as table 3 except that it is designed to facilitate an analysis of the differences between the *BrBr* and *Brbr* segregates, the *BrBr* and *brbr* segregates, and the *Brbr* and *brbr* segregates.

From Table 3, it can be seen that in every case the *vv* plants gave a larger yield of seed per plant than did the *Vv* or *VV* segregates and *Vv* segregates exceeded *VV* segregates. The lowest t value obtained for any of these comparisons was 5.495 which gives odds greater than 99:1 against the deviations noted being due to the errors of random sampling. *Vv* exceeded *vv* plants among the *BrBr* and *Brbr* segregates for number of spikes per plant, height of plant, and length of awn and the t values for the differences had a P value of less than .01 in all cases. Likewise, the *Vv* plants exceeded the *vv* plants among the *brbr* progeny but the t values were less than the above; that for number of spikes per plant being .658, for height of plant 3.197, and for length of awn 1.601. It will be remembered that a value of 1.960 is necessary for a P value as low as .05. For spikes per plant, height of plant, and length of awn the *VV* segregates exceeded those possessing *vv* for the *BrBr* and *Brbr* genotypes, but the t values for this comparison in the *BrBr* genotypes were only .700 and 1.632 for number of spikes per plant and height of plant, respectively, and 2.467 for length of awn. The t values in the *Brbr* progeny for spikes per plant, height of plant, and length of awn were 2.207, 5.304 and 4.898 respectively. The reverse was true for the *brbr* segregates since the *vv* progeny exceeded the *VV* progeny. The t values were 4.268, 1.968 and 4.268 which are sufficiently large to give substantial odds against the differences noted being chance deviations due to the errors of random sampling.

Table 4 shows that in respect to weight of seed per plant *Brbr* exceeded *BrBr* in two of the three comparisons, namely, in the *VV* and *vv* segregates. The odds against the difference being due to the errors of random sampling in the first case were a trifle less than 99:1 and in the second

TABLE 3

The comparative effect upon four quantitative characters of genes differentiating and associated in inheritance with type of spike.

PHENOTYPE AND GENOTYPE	WEIGHT OF SEED PER PLANT IN GRAMS			SPIKES PER PLANT			HEIGHT OF PLANT IN INCHES			LENGTH OF AWN IN MILLIMETERS		
	WEIGHT		DIFFERENCE	NUMBER		DIFFERENCE	HEIGHT		DIFFERENCE	LENGTH		DIFFERENCE
Vulgare (*vv*) vs. deficiens (*Vv*)												
Normal (*BrBr*)	7.3	5.1	2.22	5.7	7.4	−1.72	25.9	27.4	−1.54	127.3	143.8	−16.58
Normal (*Brbr*)	8.0	5.0	2.98	5.6	6.6	−.94	25.3	27.3	−2.08	126.3	142.7	−16.42
Brachytic (*brbr*)	4.5	2.6	1.88	4.8	4.9	−.13*	17.2	18.3	−1.03	62.7	64.7	− 1.95*
Vulgare (*vv*) vs. deficiens (*VV*)												
Normal (*BrBr*)	7.3	3.9	3.44	5.7	5.9	− .21*	25.9	26.5	− .64*	127.3	134.8	− 7.50†
Normal (*Brbr*)	8.0	4.2	3.74	5.6	6.0	− .39*	25.3	26.7	−1.41	126.3	135.5	− 9.20
Brachytic (*brbr*)	4.5	1.7	2.74	4.8	3.8	.94	17.2	16.5	.68†	62.7	56.0	6.68
Deficiens (*Vv*) vs. deficiens (*VV*)												
Normal (*BrBr*)	5.1	3.9	1.22	7.4	5.9	1.51	27.4	26.5	.90	143.8	134.8	9.08
Normal (*Brbr*)	5.0	4.2	.76	6.6	6.0	.55	27.3	26.7	.67†	142.7	135.5	7.22
Brachytic (*brbr*)	2.6	1.7	.86	4.9	3.8	1.07	18.3	16.5	1.71	64.7	56.0	8.63
B1 parent (*VVBrBr*) vs. F_2 (*VVBrBr*)	1.9	3.9	−2.00	3.7	5.9	−2.2	25.1	26.5	−1.4	137.8	134.8	3.00*
Brachytic parent (*vvbrbr*) vs. F_2 (*vvbrbr*)	4.00	4.5	− .50*	4.7	4.8	− .10*	16.1	17.2	−1.1	71.0	62.7	8.3
B 1 parent (*VVBrBr*) vs. B 1 parent (*VVBrBr*)	1.9	1.9	.00	3.8	3.7	.10*	25.6	25.1	.5*	141.6	137.8	3.8*

* t test gives P > .05.

† t test gives P < .05 but > .01.

TABLE 4

The comparative effect upon four quantitative characters of genes differentiating and associated in inheritance with habit of growth.

PHENOTYPE AND GENOTYPE	WEIGHT OF SEED PER PLANT IN GRAMS			SPIKES PER PLANT			HEIGHT OF PLANT IN INCHES			LENGTH OF AWN IN MILLIMETERS		
	WEIGHT		DIFFERENCE	NUMBER		DIFFERENCE	HEIGHT		DIFFERENCE	LENGTH		DIFFERENCE
Normal (*BrBr*) vs. normal (*Brbr*)												
Vulgare (*vv*)	7.3	8.0	−.67*	5.7	5.6	.05*	25.9	25.3	.60*	127.3	126.3	.95*
Deficiens (*Vv*)	5.1	5.0	.09*	7.4	6.6	.83	27.4	27.3	.06*	143.8	142.7	1.11*
Deficiens (*VV*)	3.9	4.2	−.37†	5.9	6.0	−.13*	26.5	26.7	−.17*	134.8	135.5	−.75*
Normal (*BrBr*) vs. brachytic (*brbr*)												
Vulgare (*vv*)	7.3	4.5	2.82	5.7	4.8	.90	25.9	17.2	8.63	127.3	62.7	64.56
Deficiens (*Vv*)	5.1	2.6	2.48	7.4	4.9	2.49	27.4	18.3	9.14	143.8	64.7	79.19
Deficiens (*VV*)	3.9	1.7	2.12	5.9	3.8	2.05	26.5	16.5	9.95	134.8	56.0	78.74
Normal (*Brbr*) vs. brachytic (*brbr*)												
Vulgare (*vv*)	8.0	4.5	3.49	5.6	4.8	.85	25.3	17.2	8.03	126.3	62.7	63.61
Deficiens (*Vv*)	5.0	2.6	2.39	6.6	4.9	1.66	27.3	18.3	9.08	142.7	64.7	78.08
Deficiens (*VV*)	4.2	1.7	2.49	6.0	3.8	2.18	26.7	16.5	10.12	135.5	56.0	79.49

* t test gives P>.05.

† t test gives P<.05 but>.01.

case were 9:1. It seems reasonably safe to conclude that the *Brbr* segregates in the *VV* plants possessed a somewhat larger weight of seed per plant than did the *BrBr* segregates. In every case and for all four characters, the *BrBr* and *Brbr* segregates exceeded the *brbr* plants and in no comparison were the odds against the differences being due to the errors of random sampling less than 99:1.

THE NATURE OF THE INTERACTION BETWEEN THE NON-ALLELIC GENES

The nature of the interaction between the non-allelic genes is of particular interest, as it is of fundamental importance to know whether the favorable growth genes give the same increase over the less favorable growth genes in all genotypes. To facilitate the study of this problem, the data in table 5 were compiled. This table gives the interactions between the genes

TABLE 5

The interactions between those genes differentiating and associated in inheritance with type of spike and habit of growth as regards their effect upon four quantitative characters.

INTERACTION	WEIGHT OF SEED DIFFERENCE		SPIKES PER PLANT DIFFERENCE		HEIGHT OF PLANT DIFFERENCE		LENGTH OF AWN DIFFERENCE	
	GRAMS	t	NO.	t	IN.	t	MM.	t
Vulgare (*vv*) vs. deficiens (*Vv*) and								
Normal (*BrBr*) vs. normal (*Brbr*)	.76	2.183	.78	2.693	.54	1.195	.16	.058
Normal (*BrBr*) vs. brachytic (*brbr*)	.34	.992	1.59	4.993	.51	1.039	14.63	5.442
Normal (*Brbr*) vs. brachytic (*brbr*)	1.10	3.543	.81	3.039	1.05	2.446	14.47	6.227
Vulgare (*vv*) vs. deficiens (*VV*) and								
Normal (*BrBr*) vs. normal (*Brbr*)	.30	.697	.18	.553	.77	1.624	1.70	.503
Normal (*BrBr*) vs. brachytic (*brbr*)	.70	1.627	−1.15	3.000	−1.32	2.484	−14.18	3.940
Normal (*Brbr*) vs. brachytic (*brbr*)	1.00	2.448	−1.33	4.503	−2.09	4.476	−15.88	5.486
Deficiens (*Vv*) vs. deficiens (*VV*) and								
Normal (*BrBr*) vs. normal (*Brbr*)	.46	2.143	.96	3.676	.23	.542	1.86	.697
Normal (*BrBr*) vs. brachytic (*brbr*)	.36	1.742	.44	1.524	.81	1.631	.45	.160
Normal (*Brbr*) vs. brachytic (*brbr*)	.10	.469	.52	1.966	1.04	2.317	1.41	.581

differentiating and associated by linkage with type of spike and those differentiating and associated by linkage with habit of growth. A brief statement concerning the formulation of this table will help to clarify the discussion which follows. For illustration consider the interaction *vv* vs. *Vv* and *BrBr* vs. *Brbr*. The information given is whether the difference between *vv* and *Vv* in combination with *BrBr* is the same as in combination with *Brbr*. This may be tested by comparing the two differences. Thus the difference $(vvBrBr - VvBrBr) - (vvBrbr - VvBrbr)$ for weight of seed is .76 grams, as can be seen from table 5 in which these differences between two differences are listed together with the t values for testing their significance. If the difference $(vvBrBr - VvBrBr) - (vvBrbr - VvBrbr)$ is statistically significant, then it must necessarily follow that the difference

(*vvBrBr* − *vvBrbr*) − (*VvBrBr* − *VvBrbr*) must also be statistically significant, as the final figures are identical. The reason for this is apparent as in both cases we are dealing with the same interaction stated differently. The former statement emphasises the *Vv* genes whereas the latter places the emphasis on the *Brbr* genes. In the discussion of the interactions in this article, for the sake of clarity emphasis will be placed on the *Vv* genes.

From the t values in table 5, it can be readily determined which interactions are statistically significant. The following interactions for weight of seed have t values larger than 1.960; *vv* vs. *Vv* and *BrBr* vs. *Brbr*, *vv* vs. *Vv* and *Brbr* vs. *brbr*, *vv* vs. *Vv* and *Brbr* vs. *brbr*, *vv* vs. *VV* and *Brbr* vs. *brbr*, and *Vv* vs. *VV* and *Brbr* vs. *BrBr*. Considering spikes per plant, only two of the nine interactions have t values lower than 1.960; whereas, both height of plant and length of awn have five t values below this figure. These data are conclusive in showing that the interactions between the non-allelic genes are of such a nature that the more favorable growth factors do not give the same increase over the less favorable growth factors in all genotypes.

The nature of this interaction as determined by which genotype induces larger increases can be determined from the data in tables 3, 4, and 5. Table 3 shows that *vv* plants combined with a high producing genotype are favored more than are *Vv* or *VV* plants, as concerns weight of seed per plant. For example, the difference between *vv* and *Vv* plants in the *BrBr* genotype is 2.22, in *Brbr* is 2.98, and in *brbr* is only 1.88. Likewise, the difference between *vv* and *VV* plants in the *BrBr* genotype is 3.44, in *Brbr* is 3.74, and in *brbr* is only 2.74 grams. From the t tests of the interactions given in table 5, it can be seen that 2.98 is significantly different from 2.22 and 1.88 and that 3.74 is significantly different from 2.74. Table 3 shows that the difference between *Vv* and *VV* plants was greater in the *BrBr* genotype than it was in *Brbr* or *brbr* and the t test for the interactions listed in table 5 show that this difference involving the *BrBr* and *Brbr* genotypes is probably statistically significant. Therefore, in this study, it is evident that the genes favorable to high plant yields when transferred from a low to a high yielding genotype are, in comparison with their alleles and in absolute values, still more favorable to plant growth. The only exceptions to this were the difference between the *Vv* and *VV* plants in the *Brbr* genotype in comparison with the difference between the *Vv* plants and the *VV* plants in the *brbr* genotype. The differences were obtained from means of 5.0 and 4.2 grams in comparison with 2.6 and 1.7 grams. In this case the smaller means did not give the smaller difference; that is, the *Vv* plants in comparison with the *VV* plants did not give an increased difference in the higher yielding genotypes.

It remains to be seen whether the same general conclusions can be drawn

for the other three quantitative characters studied. In making the comparison between *vv* and *Vv* plants for the *BrBr* and *Brbr* genotypes. it is necessary to know whether the latter two genotypes differ. The only statistically significant difference between these two genotypes, as can be seen from table 4, was for spikes per plant in the *Vv* genotype. Then, if the more favorable genes give still greater increases when in the higher tillering genotypes, it would be expected that the difference between *vv* plants and *Vv* plants would be still greater in the *BrBr* genotype than the same comparison in the *Brbr* genotype. That such actually was the case can be seen from table 3, and that the difference between the two differences of −1.72 and −.94 was significant can be seen from table 5. The t value is larger than necessary to give odds of 99:1 against the deviation of .78 being due to the errors of random sampling. Likewise, according to the hypothesis that the genes more favorable to growth give greater increases over those less favorable in combination with genes also favorable to growth, the difference between *vv* and *Vv* plants of the genotypes *BrBr* and *Brbr* should be greater than the difference between *vv* and *Vv* plants in the *brbr* genotype. Such was found to be the case for all characters (see table 3) and with the exception of the comparison between the differences involving *vv* and *Vv* in the *BrBr* and *brbr* genotypes for height of plant the differences were statistically significant.

In comparing the *vv* and *VV* plants the *BrBr* and *Brbr* genotypes may be omitted as the latter two genotypes do not differ significantly in the three quantitative characters measured. The hypothesis that the differences would be still greater in the genotypes of higher values does not fit the data as regards the comparison between *vv* and *VV* plants in the genotypes *BrBr* and *Brbr* compared with *brbr*. For example, in the *brbr* genotype *vv* plants have a greater number of spikes per plant, a greater height and a longer awn; whereas, in the *Brbr* genotype the reverse is true and in the *BrBr* genotype the only significant difference is for length of awn and it is in favor of the *VV* plants. As regards the comparison between *Vv* and *VV* plants and involving the three characters spikes per plant, height of plant, and length of awn, the only consistent difference seems to be that in every case the *Brbr* genotype gave lower differences (table 3) than did the *BrBr* and *brbr* genotypes. The differences noted for the interaction *Vv* vs. *VV* and *BrBr* vs. *Brbr* for number of spikes were statistically significant as were also the differences for the interaction *Vv* vs. *VV* and *Brbr* vs. *brbr* for number of spikes and height of plant. The differences are given in tables 3 and 4 and the interaction in table 5.

The regression coefficients of weight of seed on number of spikes give additional information concerning the nature of the interactions of the genes influencing yield of seed per plant. Table 6 shows that in every case

the addition of a spike among the *vv* segregates gave a greater increased yield than did an addition of a spike among the *Vv* or *VV* segregates. Likewise an addition of a spike per plant gave a greater increase in yield among the *Vv* segregates than did the addition of a spike per plant among the *VV* segregates. The difference between these two genotypes is statistically significant in two out of the three comparisons. Turning to the different combinations of *Br* and *br*, the *Brbr* plants gave a greater increased yield for each additional spike per plant than did the *BrBr* plants, (see table 7). In only one case however, did the odds against the differences being due to the errors of random sampling exceed 19:1. In every case an

TABLE 6

The regression coefficients of weight of seed on number of spikes compared on the basis of genes associated in inheritance with type of spikes.

PHENOTYPE AND GENOTYPE	WEIGHT OF SEED PER PLANT ON NUMBER OF SPIKES			
	REGRESSION COEFFICIENTS		DIFFERENCE	t
	grams	*grams*	*grams*	
Vulgare (*vv*) vs. deficiens (*Vv*)				
Normal (*BrBr*)	1.03	.73	.30	3.121
Normal (*Brbr*)	1.40	.78	.62	8.448
Brachytic (*brbr*)	.82	.56	.26	2.555**
Vulgare (*vv*) vs. deficiens (*VV*)				
Normal (*BrBr*)	1.03	.63	.40	3.348
Normal (*Brbr*)	1.40	.69	.71	7.490
Brachytic (*brbr*)	.82	.51	.31	2.297**
Deficiens (*Vv*) vs. deficiens (*VV*)				
Normal (*BrBr*)	.73	.63	.10	1.995**
Normal (*Brbr*)	.78	.69	.09	1.942*
Brachytic (*brbr*)	.56	.51	.05	.915*
B1 parent (*VVBrBr*) vs. F_2 genotype (*VVBrBr*)	.53	.63	−.10	1.968**
Brachytic parent (*vvbrbr*) vs. F_2 genotype (*vvbrbr*)	.70	.82	−.12	.870*
B1 parent (*VVBrBr*) vs. B1 parent (*VVBrBr*)	.54	.53	.01	.142*

* t gives P>.05 ** t gives P<.05 but >.01.

additional spike gave a greater increase per plant in the *BrBr* segregates than was the case as regards the *brbr* segregates. Here again, only one of the differences was statistically significant but it was decidedly so. In all cases the *Brbr* segregates gave a greater increased yield per additional spike than was true for the *brbr* segregates and all the differences were statistically significant.

It is of interest to know the manner of interaction that exists among the genes affecting yield. For example, do *vv* plants give the same increase per additional spike over *Vv* plants, whether in combination with *BrBr*, *Brbr*, or *brbr*? It is evident from table 6 that the increase is greater in combination with *Brbr* than when in combination with *BrBr* or *brbr*. The same is

true for the comparison between the *vv* and *VV* plants. The t values for these interactions were found to exceed 1.96. It is evident that a difference in interaction of factors exists, and the only general conclusion to be drawn, and which does not hold for all cases, is that genes favorable to increased yield per additional spike give still greater absolute increased yields when in combination with other genes which also favor the development of this character.

TABLE 7

The regression coefficients of weight of seed on number of spikes compared on the basis of genes associated in inheritance with habit of growth.

PHENOTYPE AND GENOTYPE	WEIGHT OF SEED PER PLANT ON NUMBER OF SPIKES			
	REGRESSION COEFFICIENTS		DIFFERENCE	t
	grams	*grams*	*grams*	
Normal (*BrBr*) vs. Normal (*Brbr*)				
Vulgare (*vv*)	1.03	1.40	−.37	2.459**
Deficiens (*Vv*)	.73	.78	−.05	1.200*
Deficiens (*VV*)	.63	.69	−.06	1.260*
Normal (*BrBr*) vs. brachytic (*brbr*)				
Vulgare (*vv*)	1.03	.82	.21	.994*
Deficiens (*Vv*)	.73	.56	.17	3.934
Deficiens (*VV*)	.63	.51	.12	1.853*
Normal (*Brbr*) vs. brachytic (*brbr*)				
Vulgare (*vv*)	1.40	.82	.58	3.010
Deficiens (*Vv*)	.78	.56	.22	4.512
Deficiens (*VV*)	.69	.51	.18	3.089

* t gives P>.05 ** t gives P<.05 but >.01.

COMPARISON BETWEEN THE F_1 GENERATION, CERTAIN F_2 GENERATION GENOTYPES AND THE PARENTS

The comparison of the F_1 generation and parents and certain genotypes of the F_2 generation and parents provides information concerning the effect upon weight of seed per plant of genes not linked with the qualitative characters analyzed in this study (table 8). It will be remembered from the discussion of methods that due to the fact that F_1 and F_2 generations were grown some distance apart, although in the same series, a comparison between them must be made by use of the parents. The difference in yield between the F_1 generation *VvBrbr* and the B 1 parent *VVBrBr* was 4.1 grams; whereas, the difference in yield between the F_2 generation plants *VvBrbr* and the B 1 parent *VVBrBr* plants was 3.1 grams (table 8). Both of these differences have a P value of less than .01 and therefore, are undoubtedly statistically significant. The difference between the two differences also shows P<.01. This means that the difference between F_1 *VvBrbr* and the B 1 parent was greater than the difference between F_2

VvBrbr and B 1 parent. The same relationship held for the comparison between F_1 *VvBrbr* and F_2 *VvBrbr* plants with the Brachytic parent plants, but the difference between the two differences was not statistically significant. These data show that there must have been a reduction in weight of seed per plant between F_1 (*VvBrbr*) and F_2 (*VvBrbr*) plants. This reduction can be accounted for by partially dominant genes affecting yield and at least not closely linked with *Vv* or *Brbr*, probably the ones having the most effect being independently inherited.

The presence of these genes may be tested further by comparing the F_2 plants of the genotypes *VVBrBr* and *vvbrbr* with the parental plants of the same genotypes, respectively (see table 8). If there are no genes other

TABLE 8

Weight of seed per plant of the parents compared with weight of seed per plant of the F_1 and of certain F_2 genotypes.

COMPARISON	N	WEIGHT OF SEED PER PLANT IN GRAMS			
		WEIGHT		DIFFERENCE	t
F_1 (*VvBrbr*) vs. B1 parent (*VVBrBr*)	57 and 50	5.6	1.5	4.1	7.930
F_1 (*VvBrbr*) vs. brachytic parent (*vvbrbr*)	57 and 60	5.6	4.3	1.3	2.012**
F_2 (*VvBrbr*) vs. B1 parent (*VVBrBr*)	249 and 266	5.0	1.9	3.1	34.171
F_2 (*VvBrbr*) vs. brachytic parent (*vvbrbr*)	249 and 63	5.0	4.0	1.0	3.229
F_2 (*VVBrBr*) vs. B1 parent (*VVBrBr*)	78 and 266	3.9	1.9	2.0	28.189
F_2 (*vvbrbr*) vs. brachytic parent (*vvbrbr*)	64 and 63	4.5	4.0	.5	.761*
F_1 (*VvBrbr*) vs. B1 parent (*VVBrBr*) and F_2 (*VvBrBr*) vs. B1 parent (*VVBrBr*)				1.0	4.148
F_2 (*VVBrBr*) vs. B1 parent (*VVBrBr*) and F_2 (*vvbrbr*) vs. brachytic parent (*vvbrbr*)				1.5	5.807

* t test gives P > .05.

** t gives P < .05 but > .01.

than those closely linked with *Vv* and *Brbr* which affect yield and are segregating, the differences in yield between parental and F_2 plants of the corresponding genotype should not be statistically significant. Table 8 shows that the difference between F_2 plants *VVBrBr* and the B 1 parent *VVBrBr* is statistically significant, whereas that between the F_2 *vvbrbr* plants and the Brachytic parent *vvbrbr* is not. Also, the difference betwen the two differences is statistically significant. It is evident that the genes more favorable to high yield in the F_2 plants of the genotype *VVBrBr* than in F_2 plants of the genotype *vvbrbr* must have entered the cross from the Brachytic parent. There is no corresponding depressing effect on yield of grain per plant of the alleles of these genes entering the cross from the B 1 parent; if there were, the F_2 *vvbrbr* segregates should yield less than the Brachytic parent *vvbrbr*. Such was not the case. Again there is proof of a difference in the interaction of factors.

THE VARIANCES OF THE DIFFERENT GENOTYPES AND PARENTS

When studying quantitative characters, it has been the practice of geneticists to use homozygous material as a measure of the environmental variability. By the analysis of variance, it is possible to divide the total variability into that due to the genes isolated in the population being studied and that due to residual genetic variability plus environmental variability. In certain cases it is desirable to determine the residual genetic variability. If the parents can be used to measure the environmental variability, it can be accomplished readily by analyzing the variability into that due to genotypes and within genotypes. The variability within genotypes minus the environmental variability which has been measured by the parents would leave the residual genic variability. This method has been used by RASMUSSON (1935).

TABLE 9

The means and non-adjusted variances for the different phenotypes and genotypes.

PHENOTYPE	GENOTYPE	WEIGHT OF SEED IN GRAMS		NUMBER OF SPIKES PER PLANT		HEIGHT OF PLANT IN IN.		LENGTH OF AWN IN MM.	
		MEAN	VARIANCE	MEAN	VARIANCE	MEAN	VARIANCE	MEAN	VARIANCE
Vulgare normal	(*vvBrbr*)	8.0	19.289	5.6	6.562	25.3	5.705	126.3	237.516
Vulgare normal	(*vvBrBr*)	7.3	16.625	5.7	8.157	25.9	6.704	127.3	384.996
Deficiens normal	(*VvBrBr*)	5.1	7.744	7.4	12.240	27.4	7.323	143.8	265.903
Deficiens normal	(*VvBrbr*)	5.0	7.518	6.6	9.578	27.3	7.067	142.7	226.634
Vulgare Brachytic	(*vvbrbr*)	4.5	5.224	4.8	2.880	17.2	3.824	62.7	73.577
Deficiens normal	(*VVBrbr*)	4.2	4.646	6.0	8.194	26.7	4.726	135.5	239.844
Deficiens normal	(*VVBrBr*)	3.9	5.359	5.9	10.467	26.5	5.837	134.8	320.472
Deficiens Brachytic	(*Vvbrbr*)	2.6	2.588	4.9	6.106	18.3	5.834	64.7	75.796
Deficiens Brachytic	(*VVbrbr*)	1.7	1.560	3.8	4.858	16.5	5.536	56.0	121.754
B 1 Parent	(*VVBrBr*)	1.9	1.668	3.7	4.553	25.1	9.224	137.8	241.487
Brachytic Parent	(*vvbrbr*)	4.0	7.693	4.7	7.426	16.1	2.027	71.0	47.822
B 1 Parent	(*VVBrBr*)	1.9	1.690	3.8	4.641	25.6	6.617	141.6	163.416

Table 9 gives the variances and means for the different genotypes and parents. It should be recalled that the variances found for the Brachytic and B 1 parents listed in the last two rows of table 9 are not directly comparable with these given for the F_2 genotypes and B 1 parent, as they were grown in rows at the end of the series, whereas the data from the nine B 1 families, distributed at random with the twenty F_2 families would be comparable with the data from the F_2. The Brachytic parental data would be comparable with the F_2 data by means of the B 1 parent.

As regards weight of seed per plant, it is apparent that all genotypes are not equally variable. The genotypes with the larger means also have the larger variances. This is equally true for the parents. The relationship is not so close for the number of spikes per plant, height, and length of awn, but it does exist.

The comparison between the parents and F_1 plants furnishes additional evidence. In this study the mean weight per plant of the B 1 parent was 1.5 grams and the variance 1.379, for the Brachytic parent was 4.3 grams and the variance 6.219 and for the F_1 the mean was 5.6 grams and the variance 10.526.

It seems advisable to determine whether genotypes having means of similar magnitude have variances of different magnitudes. The means of the *Brbr* segregates for all four quantitative characters are similar to the means of the *BrBr* segregates. To ascertain whether differences in variability existed, the variances were obtained for all of the *Brbr* and *BrBr* segregates. FISHER'S Z test (1934, p. 216) was applied. It was found that the variance for the *BrBr* plants was larger than the variance for the *Brbr* segregates for number of spikes and length of awn. The values obtained by dividing Z by its standard error were 2.260 and 2.624 respectively. It appears that all genotypes do not have the same variance even though their means may be of similar magnitude.

These results make it apparent that in studies such as this, erroneous conclusions are likely to be drawn in estimating the residual genic variability, by use of parental data as an absolute measure of environmental variation, as the amount of variation due to the environment is not the same for all genotypes.

THE NATURE OF THE INTERACTION BETWEEN THE GENES AFFECTING WEIGHT OF SEED PER PLANT AND THE ENVIRONMENT

It was found that in general the genes more favorable to higher yields of seed per plant gave still higher absolute yields in comparison with their alleles when in combination with high yielding genotypes than when in combination with low yielding genotypes. This raises the question as to whether the same relationship might not exist in a comparison involving different environments.

To obtain evidence the population for each of the nine genotypes was divided into three levels of yield by the use of the normal curve. The levels thus established within reasonable limits gave the same number of individuals in each class. This method of division does not eliminate the residual genic variability as genes not closely linked and those inherited independently of those identified in this study would have an effect as well as environmental conditions. Thus some of the plants falling in the upper 1/3 may be in that range partially because of favorable genes. An estimate of the importance of the effect of the residual genic variability can be obtained by a comparison of the weight of seed per plant for the parents and the F_2 segregates of the same genotype. The comparison is given in table 3. The B 1 parent (*VVBrBr*) yielded 2 grams less than the F_2 plants

of the genotype *VVBrBr* and the Brachytic parent (*vvbrbr*) yielded .5 of a gram less than the F_2 plants of the genotype *vvbrbr*. From table 10, it can be seen that the range in yields of any one genotype for the different levels is considerably greater than the above. Therefore, from these data it appears that the environment played the most important part in determining into which level of yield a plant of a given genotype would fall.

The three levels of weight of seed per plant are listed in table 10. The difference between combinations of *Vv* and the differences between the combinations of *Brbr* are listed in table 11. As in all the previous studies these comparisons include the effect upon weight of seed per plant of the genes linked with *Vv* and *Brbr* as well as the effect of these genes themselves. The completeness with which the effect of the linked genes is meas-

TABLE 10

Weight of seed per plant of the different genotypes for three levels of yield.

PHENOTYPE	GENOTYPE	LEVEL OF YIELD		
		UPPER	MIDDLE	LOWER
Vulgare normal	(*vvBrBr*)	12.1	7.2	3.2
Vulgare normal	(*vvBrbr*)	13.5	7.9	4.1
Vulgare brachytic	(*vvbrbr*)	6.9	4.4	2.1
Deficiens normal	(*VvBrBr*)	8.7	5.2	2.3
Deficiens normal	(*VvBrbr*)	8.1	4.9	2.3
Deficiens brachytic	(*Vvbrbr*)	4.5	2.4	1.1
Deficiens normal	(*VVBrBr*)	6.9	3.9	1.4
Deficiens normal	(*VVBrbr*)	6.8	4.1	2.2
Deficiens brachytic	(*VVbrbr*)	3.3	1.6	.7

ured depends of course upon the closeness of the linkage relationship. It is apparent from table 11 that, with the possible exception of *BrBr* compared with *Brbr* in combination with *VV*, the more favorable the environmental conditions the greater in absolute values the spread between the genes more favorable to yield and those less favorable to yield. Also, as noted from the previous data, the differences are greater for the higher yielding genotypes.

DISCUSSION AND CONCLUSIONS

Recently geneticists have become interested in the nature of the interaction of factors governing the inheritance of quantitative characters. Of interest in this respect is RASMUSSON'S (1935) interaction hypothesis which assumes "that the effect of each factor on the genotype is dependent upon all the other factors present, the visible effect of a certain factor being smaller the greater the number of factors acting in the same direction."

RASMUSSON found support for his hypothesis in a study on the interaction of factors governing early and late maturity in Pisum. POWERS (1934) in studying the inheritance of habit of growth in Triticum obtained results which would support this hypothesis also. However, the nature of the interaction of the factors affecting weight of seed per plant, number of spikes per plant, height of plant and length of awn was, generally speaking, quite the reverse of that expected on the above hypothesis in that the effect of certain factors was not smaller the greater the number acting in a certain direction.

TABLE 11

Differences in weight of seed per plant for three levels of yield.

GENOTYPE			LEVELS OF YIELD		
			UPPER	MIDDLE	LOWER
vv	vs.	*Vv (BrBr)*	3.4	2.0	.9
vv	vs.	*VV (BrBr)*	5.2	3.3	1.8
Vv	vs.	*VV (BrBr)*	1.8	1.3	.9
vv	vs.	*Vv (Brbr)*	5.4	3.0	1.8
vv	vs.	*VV (Brbr)*	6.7	3.8	1.9
Vv	vs.	*VV (Brbr)*	1.3	.8	.1
vv	vs.	*Vv (brbr)*	2.4	2.0	1.0
vv	vs.	*VV (brbr)*	3.6	2.8	1.4
Vv	vs.	*VV (brbr)*	1.2	.8	.4
BrBr	vs.	*Brbr (vv)*	−1.4	−.7	−.9
BrBr	vs.	*brbr (vv)*	5.2	2.8	1.1
Brbr	vs.	*brbr (vv)*	6.6	3.5	2.0
BrBr	vs.	*Brbr (Vv)*	.6	.3	.0
BrBr	vs.	*brbr (Vv)*	4.2	2.8	1.2
Brbr	vs.	*brbr (Vv)*	3.6	2.5	1.2
BrBr	vs.	*Brbr (VV)*	.1	−.2	−.8
BrBr	vs.	*brbr (VV)*	3.6	2.3	.7
Brbr	vs.	*brbr (VV)*	3.5	2.5	1.5

The logical conclusion to be drawn from these data is that the nature of the interaction of the genes affecting the quantitative characters is sufficiently variable to render any hypothesis of doubtful value as a means of prediction. From our more extensive knowledge of the interaction of factors governing the inheritance of quantitative characters it is not surprising that this is the case. In fact, BRINK (1934) found that the interaction of factors governing anthocyanin plant colors in maize was such that plants of the genotypes *AbPl* and *ABpl* averaged higher in dry weight of ears per plant than did plants of the genotypes *ABPl* and *Abpl*. It seems from these

results and others reported here that the nature of the interaction of factors affecting quantitative characters is sufficiently variable to require extensive genetic studies involving a large variety of characters and organisms before a hypothesis of much value for prediction purposes can be formulated and then it may be expected to be rather limited in application.

EAST (1935) develops a concept concerning genes that is useful in the interpretation of any studies on quantitative inheritance. He divides gene mutations into two broad classes: physiological defectives and physiological non-defectives. These are discussed in connection with the bearing that they have upon evolution. Physiological defective gene mutations comprise the great bulk of those found in the genetic laboratory, usually are recessive to the wild type and cause restrictions in the physiological processes in which they are involved. This would mean that they may have both quantitative and qualitative effects and are easily detectable. Because of the comparative ease with which they are detected the effect must be pronounced. The non-defective gene mutations are frequent in nature but are difficult to detect individually and may show either an approach to dominance or to recessiveness. The effect of any one gene resulting as a non-defective mutation must be small.

The importance of this conception of the two broad types of gene mutations for a better understanding of the genes differentiating quantitative characters is obvious. It can be expected that the characters depending upon physiological defective genes for their expression will be differentiated from their alleles by comparatively few factor pairs; whereas, larger differences involving non-defective genes would be expected to be differentiated by a large number of gene pairs, because as is pointed out by EAST (1935) the effect of any one pair of non-defective genes is small.

The results from the present study offer some evidence for the conception that genes having an effect upon quantitative characters may be grouped into the classes noted above. The *brbr* genes which cause brachytic habit of growth would certainly be classed as physiological defective, as would the *VV* genes which produce *deficiens* type of spike. Plants possessing *VV* genes do not have kernels developed in the rudimentary lateral florets. The heterozygote *Vv* approaches the *deficiens* parent in that no lateral grains are produced, but it is distinguishable from the *VV* homozygote in that the lateral florets although rudimentary are noticeably developed. Undoubtedly the effects of the *Brbr* and *Vv* genes are both quantitative and qualitative. The genes affecting the four quantitative characters and associated with color of glume are of the non-defective type. In heterosis at least two factor pairs are necessary to account for the increases noted in the heterozygote, since the differences between the two homozygotes are not statistically significant (table 2). It seems highly probable

that even larger numbers of factor pairs than these are operating to produce the results obtained. For all four characters the *Vv* plants exceeded the *VV* plants. Again the most probable explanation seems to be that a number of partially dominant linked factors are responsible for the differences between the two genotypes. As regards the genes associated with color of glumes, the effect of any one factor pair must necessarily be small, and as EAST (1935) has pointed out such small differences are difficult to detect.

ROBERTSON AND AUSTIN (1936) studied homozygous and heterozygous green plants of *Hordeum vulgare* from families segregating for the single factor pairs $X_c x_c$ and $A_c a_c$ and found statistically significant differences in favor of the heterozygous plants for the following characters: average length of head, total number of grains per plant, and total weight of grain per plant. Again the differences were small and may be due to a number of physiologically non-defective genes. The residual genic variability noted in the study reported in this article is due probably to factors of the same nature (non-defective) as the writer failed to find any qualitative expression of them. That these genes did not react alike in all genotypes was shown by the fact that greater increases in weight of seed per plant were obtained when combined in plants of the genotype *VVBrBr* than when combined in plants of the genotype *vvbrbr*. These results show that both groups of genes—physiological defective and non-defective—do not necessarily react alike in all genotypes.

It should be pointed out that the terms physiological defective and non-defective are used by EAST in reference to gene mutations, but it is apparent that his theorems are important in a study of the inheritance of quantitative characters. Undoubtedly these two classes grade into each other and exceptions exist, but this does not vitiate the value of such a classification. It is apparent that the above conception is very useful to the workers in the field of applied genetics. Rapid strides can be made by the breeder when dealing with the physiological defective genes, whereas, when non-defective genes are involved, the advantage gained by recombining several desirable factor pairs may be expected to be small and the progeny possessing them difficult to select because of the limited number in large populations. This does not mean that the non-defective genes are not important in a breeding program, but only that they are more difficult to work with.

SUMMARY

1. A method involving the analysis of variance and co-variance was used in reducing the data.

2. The nature of the interactions of genes was such that no general rule could be drawn. However, with one exception, which was not well estab-

lished statistically, the genes favorable to high weight of seed per plant gave as great or greater differences over their alleles in combination with genes for higher yield than they did in combination with genes for lower yield. The same general behavior was noted for the genes affecting number of spikes per plant, height of plant, and length of awn, but cases were found in which plants of a given genotype surpassed plants possessing their alleles in some combinations and were surpassed by plants possessing identical alleles in other combinations.

3. The genic variability not associated with any of the three chromosome groups identified by genes having qualitative effects was found to give a greater increased weight of seed per plant when in combination with *VVBrBr* than when in combination with *vvbrbr*. Here, again, is proof of a difference in the interaction of factors.

4. It was found that genotypes affecting the same character may have different variances even though their means may be of similar magnitude.

5. It was found that the more favorable the environmental conditions the greater in absolute values would be the spread between the genes more favorable to yield and those less favorable to yield.

6. The nature of the interaction of the factors affecting weight of seed per plant, number of spikes per plant, height of plant, and length of awn was generally speaking quite the reverse of RASMUSSON's interaction-hypothesis which assumes that the visible effect of a certain factor is smaller the greater the number of factors acting in the same direction.

7. The genes affecting the four quantitative characters are grouped into physiological defective and non-defective according to EAST's (1935) terminology and the data offer some evidence in favor of his conception as to the nature of the effects of these genes. Both classes of genes showed that they necessarily did not give the same type of interaction in all genotypes.

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GENETIC STUDIES ON SELECTIVE SEGREGATION OF CHROMOSOMES IN SCIARA COPROPHILA LINTNER

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INTRODUCTION

THE unusual behavior of the chromosomes in Sciara has been studied in considerable detail both cytologically and genetically. Although the main facts regarding the distribution and behavior of the chromosomes (as revealed by cytological examination), have been known for some time, the genetic data have been incomplete because of the paucity of mutant characters with which to follow the history of the individual chromosomes in any one species. The present paper is designed to supply additional genetic data derived from an intensive study of *Sciara coprophila* Lintner, over a period of five years. Because of the great difficulty of securing mutant characters in Sciara, only a few have been obtained, even with the aid of X-rays, and some of these are unsatisfactory. Even the latter have been included, however, in order to make the account as complete as possible. The main results of this study were presented by SMITH (1932) but publication of the present account has been delayed in the hope of securing more and better characters—a hope that has not been realized.

Previous genetic studies on this species have dealt with one pair of autosomes (METZ 1927, truncate wings), and the sex chromosomes (METZ, ULLIAN, SCHMUCK, SMITH 1929–31). The new characters considered here make it possible to present the essential facts concerning the behavior of the remaining pairs of autosomes, as well as additional data concerning the first pair.

Before analyzing the genetic results in detail, it is necessary to review briefly certain aspects of chromosome behavior in Sciara. One feature of importance is the difference in chromosome number between the two sexes, and between the soma and germ line in each sex (METZ 1931). These differences are brought about by a process of chromosome elimination during cleavage in the developing egg (DUBOIS 1932). The typical number of chromosomes in somatic groups is eight in the female (one pair of V's, three pairs of rods); and seven in the male (one pair of V's, two pairs of rods, one single rod). These are shown in figure 1a and 1b. The male group is similar to the female group but lacks one rod, presumably a sex chromosome. There are present in the germ-line of both sexes, one, two, or three

additional chromosomes which are longer and thicker than the ordinary chromosomes. These are termed the "limited" chromosomes (fig. 1c), (METZ 1931, METZ and SCHMUCK 1931). Since they are found only in the germ-line, they cannot be studied by means of somatic characters and consequently will not be considered in this account. The evidence indicates, however, that these chromosomes are relatively empty of genes (METZ 1929), and that they are not true sex chromosomes.

Another phenomenon of special significance in the present study is the occurrence of a "monocentric" mitosis at the first spermatocyte division (METZ 1926, METZ, MOSES and HOPPE 1926, METZ 1933). This mitosis is accompanied by a selective segregation of chromosomes. During prophase at this division, the chromosomes are distributed at random throughout the nucleus. Although the chromosomes are present in pairs, no evidence of synapsis has been observed at any stage of spermatogenesis. A half spindle is formed with a single pole to which all the chromosomes are attached by "spindle fibres." Subsequently, without forming an equatorial

FIGURE 1. Diagrams showing chromosome groups in (a) female somatic cell, (b) male somatic cell, (c) germ-line of both sexes with "limited" chromosomes. In somatic cells the chromosomes are associated in pairs, while in oogonia and spermatogonia they are not.

plate, they move directly into anaphase. Both of the "limited" chromosomes go regularly toward the pole, but the others segregate in such a way that one member of each pair goes toward the visible pole and the other away from it, despite the fact that the "spindle fibres" of all extend toward the pole.

When the four retreating chromosomes reach the periphery of the cell, they are deflected in their course, as if the cell wall were a mechanical barrier, and eventually they come together in a group opposite the pole. Later they are extruded in a bud resembling a polar body, and take no further part in development. From the cytological evidence, it is clear that the chromosomes are distributed here in a definite and regular way, so that one group is left in the functional cell, and the other is discarded. The question now arises as to which chromosomes are retained and which are cast off, and what is the nature of the forces operating to produce this result.

The first evidence bearing on the problem was obtained through a study of the character "truncate wings" in *Sciara coprophila* (METZ 1927). Truncate is a recessive autosomal character, somewhat similar in appearance

to truncate in *Drosophila melanogaster*. It was found that the gene for truncate was transmitted through the females in the ordinary Mendelian fashion with random segregation. The males, on the other hand, bred as though they were homozygous for the gene received from their mothers, and did not transmit the gene received from their fathers. This fact indicated that in the case of one autosome pair segregation was selective, and that the autosome retained in the functional cell at the first spermatocyte division was regularly maternal in origin. It remained to be determined how far this behavior was characteristic of the other chromosomes of this species.

A second chromosome pair was identified when two recessive characters, swollen and narrow, (METZ and ULLIAN 1929, METZ and SCHMUCK 1931) were found to show typical sex-linked inheritance. Later a dominant character, Wavy, (METZ and SMITH 1931) was secured, and more recently, two additional recessive characters, round and miniature, (SMITH-STOCKING, unpublished) appeared which show the same type of sex-linked inheritance. The first evidence suggested that the sex chromosome complex of the female was XX and that of the male was XY. Later however the male soma was found to contain only seven chromosomes, indicating that it has no Y chromosome. The male germ-line possesses two sex chromosomes. One of these is evidently the same as the X in the somatic cells, but the precise nature of the other is obscured by a series of phenomena which are not yet fully analyzed and which need not be reviewed here (METZ 1934). The evidence is consistent, however, with the view that these two undergo the same type of selective segregation as that shown by the autosome pair just considered.

In each of two other species of Sciara a character was found which was inherited in the same way as truncate wings, suggesting that perhaps this unusual chromosome segregation is typical of the genus (METZ 1928, METZ 1929).

The present genetic study was undertaken for the purpose of analyzing the method of segregation of all the chromosomes of one species. It has involved: (1) the securing of new mutant characters, sufficient in number to identify each pair of chromosomes, and (2) analyzing the relationship of the new characters to each other by means of linkage tests to ascertain whether all of the chromosomes observed cytologically (save the "limited" chromosomes) were accounted for genetically. *Sciara coprophila* was used since it is the most satisfactory species for laboratory purposes. Although the sex chromosomes and one pair of autosomes had been previously identified genetically, there remained two pairs of autosomes to be studied.

Because of the difficulty of securing mutant characters, it has been necessary in the present study to make use of some characters which are

inconstant and otherwise unsatisfactory. This has not only increased the task of making genetic tests, but has also necessitated presenting here a more detailed description of the experiments than would otherwise be required. An account of culture methods and breeding technique is included, since it has not been fully treated in earlier papers.

CULTURE METHODS AND BREEDING TECHNIQUE

1. *Culture medium*

Sciara is cultured in glass vials one inch wide and four inches deep. These are sterilized and filled to a depth of approximately one inch with an agar solution made by heating together equal parts of agar-agar and water. To insure a dry surface, a small amount of sterilized ground straw is sprinkled into the vials after the medium has solidified. The vials are plugged with cotton. Half pint milk bottles may be used for maintaining mass cultures.

2. *Life history and food*

The genus Sciara belongs to the group of so-called fungus gnats, some species of which inhabit mushroom beds and often become a serious menace to commercial enterprise. The adult of *S. coprophila* is small, dark, and inconspicuous.

The flies are usually cultivated in pair matings, a single female and one or more males being placed in each vial. Since it has been shown that a given female produces offspring from only one male (Moses and Metz 1928), several males are often placed with one female as a precaution against possible sterility. Copulation usually takes place soon after the flies are placed together. The sperms are stored by the female in the spermathecae and the eggs are fertilized one by one as they leave the vagina. They are deposited on the surface of the agar and hatch into small transparent larvae in about six days, at which time they must be fed.

Many types of food have been tried with varying degrees of success. The most satisfactory one found thus far is a mixture consisting of equal parts of animal-poultry yeast, powdered mushroom, and straw. The latter serves to prevent the formation of an impervious surface layer on the cultures. When small larvae are visible (usually ten days after the parent flies have been placed in the vial), a small quantity of food mixture is sprinkled on the surface. This is soon eaten and the supply must be replenished about every second day until pupation begins. Practice alone will demonstrate what quantity of food is required. In general it is better to feed sparingly rather than abundantly, for if cultures are given too much food, the larvae fail to eat all of it, and the excess remains on top of the culture as a loose mixture, to drop out when one attempts to remove the

flies that have hatched. Although the culture method is by no means perfect as yet, it is adequate and reliable for present purposes.

3. *Temperature conditions*

Sciara is resistant to cold; the only effect of low temperature seems to be a retardation of the rate of development. The larvae are very sensitive to heat, however, and 29°C is lethal if maintained more than a short time. Higher temperatures are immediately lethal. In the laboratory the cultures are kept in an incubator with a temperature range of 22°–24°C. Moisture conditions are regulated by placing a large flat pan of water on the lowest shelf in front of an electric fan which is in continuous operation. Under these conditions the life cycle of *S. coprophila* occupies about a month, divided approximately as follows: egg stage 5–6 days; larva 14–15 days; pupa 3–4 days; adult 5–8 days. Twelve to fourteen successive generations may be grown in the course of a year.

4. *Breeding technique*

The type of inheritance found in Sciara necessitates certain variations from the usual breeding technique employed with other animals. *S. coprophila* is "monogenic," individual females typically giving "unisexual" progenies. (One bisexual line arose as a mutation (METZ 1931) and is being studied). Occasionally there will be one or more "exceptional" males in a female progeny, or "exceptional" females in a male progeny, in which case sib matings can be made. but usually such inbreeding is not possible and it cannot be relied on as a method of studying linkage. The precise methods employed will be given more fully in the section on linkage.

In maintaining mutant stocks in the laboratory it has not proved feasible to combine several characters in one stock as is done in Drosophila work. When this has been attempted, the stocks have lost viability, despite every care. For this reason pedigreed stocks of each line have been kept. In practically all the work pair matings are used in maintaining stocks, mutant females being out-crossed to wild type males from a wild stock every generation to keep the lines viable. Even with these precautions the mutant stocks frequently show poor viability. The wild stocks also show considerable variation in this respect. These fluctuations do not appear to be related to any immediate environmental effect, for it rarely happens that more than one stock is in poor condition at a given time, although all the flies are kept under the same conditions. Likewise fluctuations in viability are not usually associated with seasonal changes.

GENERAL ACCOUNT OF THE MUTANT CHARACTERS AND THEIR OCCURRENCE

It is exceedingly difficult to secure satisfactory mutant characters in Sciara. This is due in part to the remarkable resistance to radiation shown

by these flies, and in part to the physical characteristics which conceal all except the most obvious changes. Furthermore, the type of inheritance in this species tends to conceal recessive characters because the progenies are essentially unisexual and consequently sib matings are rare. Finally, the selective segregation occurring in the male prevents the transmission of paternal characters through the male line.

The normal rate of appearance of mutant characters in nature is very low, as witnessed by the fact that in hundreds of cultures of *Sciara coprophila* derived from five different localities and cultivated in the laboratory for a number of years in both pair matings and mass matings, only three mutant characters were found prior to 1930. All of these were recessives; two were sex-linked (swollen and narrow) and one was autosomal (truncate). During the course of the present study, two more sex-linked recessive characters (miniature and round) and two more autosomal characters (Delta, a dominant; oval, a recessive) have arisen spontaneously. Other mutant characters may have been spontaneous in origin, but since they came from lines subjected to X-ray treatments this cannot be concluded with certainty.

In an effort to increase the mutation rate, adult flies of both sexes were X-rayed. The treatments were given at the Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, New York, and at the Marine Biological Laboratory, Woods Hole, Massachusetts. In all cases a tungsten target was used and a 1 mm aluminum filter for intercepting the soft rays. In the work done at Cold Spring Harbor the dosage was determined by means of a Victoreen Dosimeter, while at Woods Hole mathematical calculation was employed.

Sciara is able to withstand very high dosages of X-rays as compared with most other organisms, and shows no effect from dosages commonly employed in Drosophila work (1,000 to 5,000 *r* units). The range of treatments found to be effective was from 7,000 to 20,000 *r* units. It is not certain that the X-ray treatments actually induced the mutations. However, mutations did occur in cultures that had been treated.

It is usually assumed that it is not profitable to work with dosages which produce greater than 50 percent sterility, but in a form in which mutation is rare and which is relatively resistant to artificial means of producing mutant characters, it is feasible to use dosages which cause complete sterility in as many as 70 percent of the cases. The percentage of sterility produced is extremely variable from time to time, even when the X-ray treatment is essentially the same and the flies are similar.

Although some of the mutant characters came from treated cultures, the genetic data make it seem probable that these as well as the other mutations represent actual gene changes rather than chromosome abnormali-

ties. The origin and history of each will be taken up in chronological order.

Each new mutant character was tested with respect to constancy, selective segregation and linkage. All the new characters described here are dominant wing peculiarities. One was found by Mrs. C. S. MAURICE, the remaining five by the writer. Their characteristics may be readily observed by examination of the photographs and comparison with the wild type wing shown in figure 3a.

In describing variations from the wild type, the terminology of wing venation used by JOHANNSEN (1909) is followed (fig. 2).

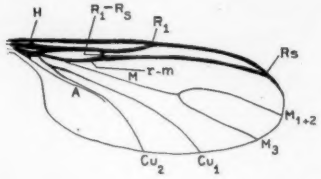

FIGURE 2. Diagram after JOHANNSEN, showing Sciara wing venation:

H = humeral cross-vein at base of wing
R = radius
R_1 = first branch of radius
R_s = posterior branch of radius
M = media
M_{1+2} = anterior branch of media
M_3 = posterior branch of media
Cu = cubitus
Cu_1 = anterior branch of cubitus
Cu_2 = posterior branch of cubitus
A = anal vein
r−m = radio-median cross vein
R_1-R_s = cross branch of radius.

1. *Curly*

a. Origin. Curly is an autosomal dominant, found April 15, 1930 in a single F_2 female from X-rayed + male. Since the character is a dominant, it is probable that it would have appeared earlier had it been the result of the X-ray treatments.

b. Description. The wing appears to have expanded normally and then become curled forward from the posterior end of the wing toward the head (fig. 3b). It is extremely variable, ranging from a barely perceptible bend in the wing to an extremely tight curl. There is no irregularity of venation or unusual pigmentation. If the curl is very extreme, the wing appears to be distorted and can be flattened out only with effort. This sometimes makes classification difficult when crosses are made involving other wing characters.

c. Occurrence. Not only is Curly variable in appearance, but it is inconstant as well; it does not always show when the gene is present. In a series of tests crossing virgin females heterozygous for Curly to wild type males from stock, the progeny from 322 pair matings were: 17,371 wild type and 8,161 Curly. That is to say, of 25,532 offspring 68 percent were wild-type

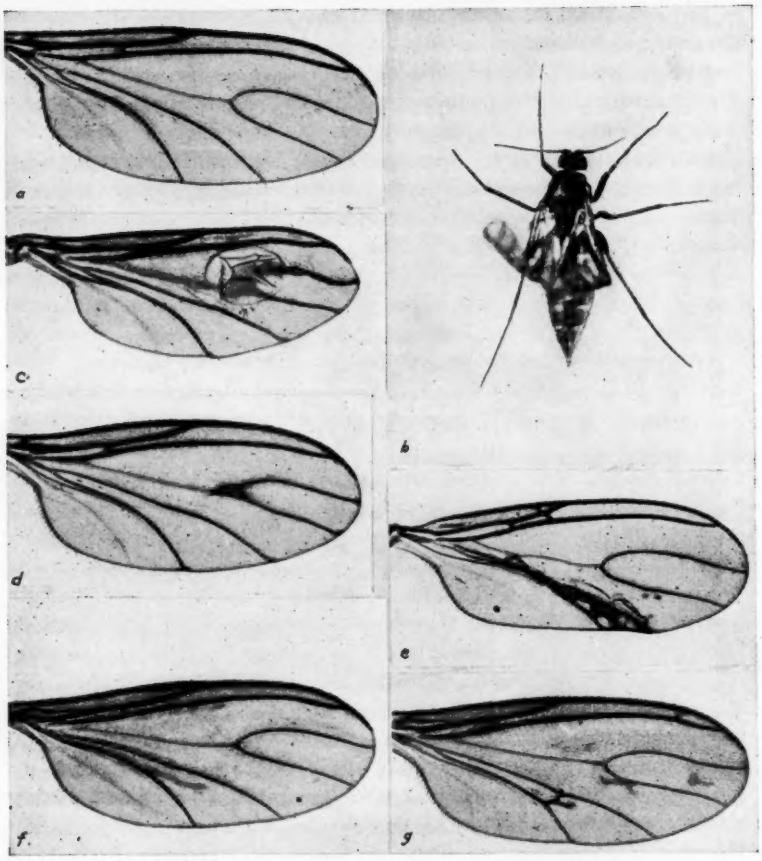

FIGURE 3. Photographs of Sciara showing:

(a) normal or wild-type wing
(b) Curly fly, whole mount
(c) Blister wing
(d) Delta wing
(e) Fused wing
(f) Dash wing
(g) Varied wing

All photographs ×35 except (b) which is ×5

and 32 percent were Curly. This deviates widely from the 1:1 ratio which would be expected if the character were constant and of normal viability.

In a test involving 467 flies (the total progeny of four pair matings tested in another connection) Curly was found to be concealed when actually present in 17.7 percent of the flies. This fact must be taken into account in analyzing linkage values.

d. Genetic behavior of the males. Since Curly is inconstant, the transmission of maternal characters through the male is correspondingly obscured. From 18 tests in which a wild type female from stock was mated to a Curly male (the son of a Curly mother), 709 wild type and 519 Curly offspring arose, whereas, all the flies would have been Curly if the character were constant. Curly was concealed in more than 57 percent of the offspring. In four of these eighteen cases, the progeny were tested further: of the 80 apparently wild-type flies (save 13 which were infertile) each gave some Curly offspring, showing that all the wild type flies were genetically Curly.

From 29 tests of Curly males which had inherited the character from their fathers, all of the offspring were wild type except three questionable flies. One of these appeared to be Curly, but on testing was found to be wild type; the other two had rumpled wings and may possibly have been Curly, but were not tested. In stock cultures flies appear occasionally which have rumpled wings but which on breeding prove to be wild type, and since this wing characteristic is transitory, it is probably an environmental effect. It is probable that the two flies not tested belong to this class. In any case the usual genetic behavior of the Curly male is typical.

2. *Blister*

a. Origin. Blister is a dominant autosomal character which appeared first May 12, 1930 in a single F_2 female from X-rayed wild type grand parents. If the character arose as the result of the X-ray treatments it must have been due to some latent effect, since the character, although a dominant, did not appear until the second generation after treatment.

b. Description. Blister appears as a large bubble in the general area of the posterior branching of the media vein (fig. 3c). In figure 2 this area is represented as the juncture of the media vein with M_{1+2} and M_3. It looks as if the wing had failed to expand normally and that an excess of liquid had collected to form this blister-like structure. The wings of any one fly are approximately symmetrical, although the size of the blister is somewhat variable. Often the whole wing is much shrunken and distorted. There is no abnormality of pigmentation and no irregularity of venation. The size of the blister varies greatly in different flies. In the extreme form the whole middle portion of the wing is involved and is pulled out of shape, but in other cases the blister may be so slight that it produces only a swell-

ing of the media vein at the point of origin of the anterior and posterior branches. In this form it is very similar to the mutation Delta.

c. Occurrence. Blister flies are of good viability and give large progenies. 29 heterozygous Blister virgin females crossed individually to + males from stock gave 1,013 + and 846 Blister. Fifteen additional matings between heterozygous Blister females and their own Blister brothers gave 486 + and 411 Blister flies. Summarizing the results: 44 heterozygous females gave 1,499 + and 1,257 Blister flies, or a proportion of about 54 percent + to 45 percent Blister. The deficiency of the Blister class probably indicates a lower viability of the mutant flies, for on the whole the average is not far from the expected 1:1 ratio.

Blister is constant and is manifest when present, as shown by the ratios and by tests of 31 wild type sisters of Blister. The latter produced a total of 4,547 flies, of which only three were noted as questionable. One of these was tested and found to be wild type; the other two were not tested.

d. Genetic behavior of the males. Blister males derived from Blister mothers transmit the character as if they themselves were homozygous for the mutant gene. In 18 crosses of wild type virgin females by Blister males of the above type, there were 1,548 offspring all Blister.

On the other hand, Blister males which inherit the character from their fathers do not transmit it. Nineteen such males were tested by crossing to wild-type virgin females from stock. The total offspring were 1,525 wild type, and 1 questionable fly which was infertile.

3. *Delta*

a. Origin. Delta is an autosomal dominant character which was found June 6, 1930. It appeared simultaneously in several flies of a mass culture from pure stock of the "bisexual" line and had no history of X-ray treatment.

b. Description. Distinguishing features are relatively constant (fig. 3d). Of these, the more obvious one is the swelling of the juncture of the media vein with its anterior and posterior branches (fig. 2). The other feature is the thickening of the marginal ends of the veins termed Cu_1, Cu_2, M_{1+2}, and M_3. Sometimes the small cross-vein ($R_1 - R_s$) is thickened noticeably. Delta is quite variable in appearance, ranging from a barely perceptible swelling of the regions designated above to a distorted, blistered wing, indistinguishable from the character Blister.

c. Occurrence. From 25 tests of heterozygous Delta females crossed to wild type males from stock, the total offspring were 1,283 + and 1,172 Delta flies. In addition, from 26 heterozygous Delta females crossed to wild type or Delta brothers which would be expected to breed pure for wild type the offspring were 1,153 wild-type and 954 Delta. Summarizing: 51 mat-

ings of heterozygous Delta females gave a total of 2,436+and 2,126 Delta flies, or approximately 53 percent+to 46 percent Delta. The mutant class showed poorer viability than the wild type.

On the whole the character is constant, although occasionally a fly that appears to be wild type is found to carry Delta. Eighteen wild type sisters of Delta females were tested in this connection. Of these, one gave a progeny of 167 wild type flies and one questionable fly which was dead when found. Another female gave a progeny of 142 wild type and 9 questionable flies, which when tested further were found to be actually Delta. The remaining 16 females gave only wild type offspring.

d. Genetic behavior of the males. Transmission of the character through the male line is typical of the usual inheritance found in Sciara; males transmit to their offspring only the genes derived from their mothers. From 10 Delta males crossed to wild type virgin females from stock, Delta being maternal in origin, only Delta offspring arose. Nine Delta males were tested which had inherited the character from their fathers; all gave only wild type offspring.

4. *Fused*

a. Origin. Fused is a dominant autosomal character which first appeared in a single female September 8, 1931. This fly was the daughter of a female which had received an X-ray treatment of 20,000 *r* units.

b. Description. The most distinctive feature of this character is the irregular fusion of the anterior and posterior branches of the cubitus vein (fig. 3e). This is always present, although the amount of fusion varies. The media vein is slightly irregular and appears to be the result of a puckering of the wing toward the lower margin, occasioned by the fusion of veins in that area. There may be a general increase in the amount of pigmentation of the whole wing, especially in the region anterior to the posterior branch of the radius vein (R_s) but this is not always present. The cross-vein ($R_1 - R_s$) may be markedly thickened. In a given fly, the wings are essentially symmetrical.

c. Occurrence. Fused flies are prolific and the character is constant. Heterozygous females, when crossed to wild type males from stock give offspring that closely approximate a 1:1 ratio. Twenty-two pair matings of this type gave 1,327+and 1,410 Fused flies, a ratio of approximately 48+ to 51 Fused. The fact that the mutant class is larger than the wild type shows the excellent viability of the mutant flies.

To test the constancy of the character, nineteen virgin wild type sisters of Fused females were crossed to wild type males from stock. These gave only wild type offspring.

An additional test was made by crossing ten wild type sisters of Fused

which were not certainly virgin (they might have mated with their wild type brother) by wild type males from stock. Nine of these gave only wild type offspring. The tenth gave only Fused offspring: 1 female and 88 males. To determine the source of Fused in these males, some were tested further; they were found to breed true for wild type. Since it is known that the males transmit only the characters derived from their mothers and these males gave only wild type offspring it is to be concluded that Fused was derived from the father, and that it was the father rather than the mother which was genetically Fused while appearing to be wild type. Apparently Fused is not often concealed for in the tests thus far made this is the only exceptional case.

d. Genetic behavior of the males. The genetic results of tests of Fused males are similar to the results obtained with other mutant characters. From 37 cases in which a wild type female from stock was crossed to a Fused male which had derived Fused from his mother, the offspring were Fused without a single exception. Likewise in 36 counts in which a wild type female was crossed to a Fused male that had received the character from his father, all of the offspring were wild type. Again the males are shown to breed as if homozygous for characters of maternal origin.

5. *Dash*

a. Origin. Dash is an autosomal dominant character which first appeared October 3, 1931. The first fly was a male, the son of a female which had been treated with 20,000 *r* units of X-rays. This male was mated successively to four different females and all the offspring showed the character. In view of further tests with this character and its transmission through the male, there can be no doubt that the mutant gene was derived from the female parent.

b. Description. The most obvious and constant characteristic is the presence of a very pronounced structure between the anterior and posterior branches of the cubitus vein (fig. 3f). This resembles the usual wing veins in color and in sharpness of outline. Its direction of slope is downward from the posterior branch of the cubitus vein toward the anterior branch. The spot is elongate and slender, variable in size, and sometimes attached at one end to the posterior branch of the cubitus vein, in which case it gives the appearance of an extra vein. More often it exists as a separate structure lying between the two veins.

Occasionally, the character is more extreme for several generations, due perhaps to the presence of undetected modifiers. This variation is characterized by the presence of numerous scattered blobs of pigment, suggesting the character Varied. Despite the altered appearance, the spot between the two branches of the cubitus vein remains distinct. Usually the Dash wing

has a cloudy appearance, with a greater amount of pigmentation in the anterior region near the wing margin.

c. Occurrence. Dash affords no exception to the usual type of inheritance found in Sciara. The character is constant and the flies are viable. 23 crosses of heterozygous Dash females by wild type males from stock, gave 1,131 + and 1,150 Dash. Seven crosses of heterozygous Dash females by wild type brothers (which would breed pure for wild type) gave a total of 282 + and 291 Dash offspring. Summarizing: 30 heterozygous females gave 1,413 wild type and 1,441 Dash flies, about 49 percent of the total being wild type and 51 percent Dash—approximately a 1:1 ratio.

d. Genetic behavior of the males. Transmission of the character through the male differs in no way from that observed previously in Sciara. Twelve Dash males which had received the character from their mothers were tested; their offspring without a single exception were Dash. Fourteen Dash males which had received the character from their fathers were tested; their offspring were all wild type.

6. *Varied*

a. Origin. Varied is a dominant, autosomal character which appeared first October 14, 1931 in one male, the son of a female which had been subjected to 13,000 *r* units of X-rays. This male was bred to several females and transmitted the character to all of his daughters and all but three of his sons. In view of the usual genetic behavior of Sciara males, the fact that practically all the offspring received the character (the three exceptional flies probably being Varied since the character has been shown to be inconstant), indicates that the mutant gene came originally from the treated female.

b. Description. This character is extremely variable in appearance. Figure 3g shows a characteristic wing. The chief feature is a general duskiness of color accompanied by scattered blobs of material resembling vein fragments. These spots are variable in size, shape, number and location. There may be several such fragments, well-distributed over the wing surface, or there may be only one, in which case it is usually confined to the region between the anterior and posterior branches of the cubitus vein. Often there is an intermittent fusion of the posterior and the first branches of the radius vein, but there is no regularity about this, either as to the amount or place of fusion. In its most simple manifestation the character looks very much like Dash, described previously. The two wings of a given fly are seldom symmetrical, although both wings usually show the same degree of modification.

c. Occurrence. Flies showing the character Varied emerge late in any culture, after the majority of the wild type flies have hatched. The division

of the hatching period into two phases is especially striking in female progenies derived from heterozygous mothers. If the number of offspring is small, it frequently happens that only the wild type flies hatch. Eighteen heterozygous Varied females crossed to wild type males from stock gave 1,082+ and 521 (or 32 percent) Varied. Eleven additional progenies were tested and the offspring which appeared to be wild type were tested further to determine their genetic constitution. The initial count was: 17 wild type (which were dead when found); 681 wild type (which were tested); 255 Varied. After testing the final result was found to be: 543 wild type and 410 Varied. The 17 dead flies and the flies which gave no offspring were classed as wild type in the final count, weighting the results in the direction of that class. Even with this possibility of error, 43 percent of the total offspring were found to be Varied. Of the 410 Varied flies in the final count, 155 (37 percent) had appeared to be wild type before being tested.

d. Genetic behavior of the males. As in the cases previously described, the males transmit only the genes of maternal origin, although Varied does not always show when the gene is present. From 25 pair matings (wild type virgin females crossed to Varied males from Varied mothers), the progeny were 151 wild type and 2,377 Varied. Although most of the offspring showed the Varied character as expected, about 6 percent appeared to be wild type. The latter were probably genetically Varied.

In testing the transmission of Varied through males which had derived the character from their fathers, 20 pair matings were made. Eighteen of these gave only wild type offspring as expected, but the remaining two showed unusual behavior. One gave only wild type offspring save for three females which appeared to be Varied (not tested). The second gave only wild type offspring save for one male which was Varied and which was crossed successively to 5 wild type virgin females from wild stock. One female gave no offspring. Three females gave female progenies in which every fly was Varied, indicating that the Varied male transmitted only the character of paternal origin. The remaining female gave 44 Varied females and 1 wild type male. (This male was tested but gave no offspring.) Many of the Varied females from these four progenies were tested; they gave progenies typical of the usual heterozygous females. Although a stock derived from this source was kept in the laboratory for almost two years, neither females nor males showed any further tendency to breed differently from flies of the regular Varied stock.

The appearance of these exceptional flies at once suggests contamination, a possibility which cannot be ruled out completely. However, if contamination had occurred through the entrance of a Varied male (Varied of maternal origin) into the cultures, as the results suggest, a larger number of Varied flies should have appeared. Each progeny consisted of more

than 100 flies, yet in the first exceptional case only three unusual flies arose, and in the second case only one.

The fact that Varied is inconstant, particularly in male progenies, further complicates the situation, but as they stand, the results suggest that this exceptional case may have come about as the result of a reverse segregation of chromosomes at the first spermatocyte division, which resulted in the retention of the chromosome of paternal origin, rather than the chromosome of maternal origin. If so, the male in this instance showed reversal of the usual type of inheritance found in Sciara.

LINKAGE

The primary purpose of the linkage tests is to identify the different chromosomes and to ascertain whether or not they all exhibit the same type of segregation. Unfortunately the type of inheritance involved here presents certain difficulties for the study of linkage because it is necessary in each case to back-cross individuals of the sex in which crossing over occurs. In spite of this difficulty, it is believed that the evidence warrants the tentative conclusion that all three autosomes have been identified.

When the present study was begun, two linkage groups were already known, one representing the sex chromsomes and the other representing the first pair of autosomes, the latter identified by the character truncate. Since none of the new characters was sex-linked, it was obvious that they did not belong to Group I.

Since backcrosses of heterozygous males are unsuitable for linkage tests because of selective segregation of chromosomes occurring during spermatogenesis, such tests had to be made in the manner indicated by the following examples. In testing the recessive character truncate with a dominant such as Blister, truncate females were crossed to Blister males (from Blister mothers). The hererozygous daughters were mated to truncate males from pure stock and the progeny counted. In testing two dominants together such as Dash and Blister, Dash females were mated to Blister males (from Blister mothers); the heterozygous daughters were outcrossed to wild type males from wild stock and the progeny were counted. Reciprocal crosses were made in both types of tests.

The results of the linkage experiments are summarized in table 1. Curly proved to be the only character representing the second pair of autosomes (Group III) and had thus to be used in spite of its inconstancy.

1. *C*×*tr*. Curly was first crossed with the recessive truncate. The two characters did not appear to be linked, but the results were obscured by the inconstancy of Curly. Since the required data were important, the tests were repeated and all the questionable flies were tested further. This entailed the testing of all wild type flies for Curly, all truncate flies for

Curly, extreme Curly flies for truncate, and shrivelled flies for both truncate and Curly. A small number of weak flies were dead soon after hatching and could not be tested further. Some of the flies that were tested proved to be infertile. In the final analysis the flies of these two groups were included in whatever class they appeared to belong.

There is no doubt some error in the final calculations due to the fact

TABLE 1

	CASES	TOTAL FLIES	PERCENTAGE CLASSIFICATION			
			+	tr	C	Ctr
1. C×tr	15	2,009	25	23	29	22
			+	B	C	BC
2. C×B	35	1,855	37	25	18	17
			+	tr	B	Btr
3. B×tr	44	3,666	29	20	43	6
			+	Δ	C	ΔC
4. C×Δ	29	2,727	29	28	18	24
			+	tr	Δ	Δtr
5. Δ×tr	35	3,985	25	25	26	22
			+	B	F	BF
6. B×F	30	2,345	3	46	46	4
			+	Δ	F	FΔ
7. F×Δ	13	650	6	35	56	.9
			+	tr	F	Ftr
8. F×tr	11	565	28	20	31	20
			+	C	F	FC
9. F×C	8	361	32	15	34	17
			+	B	D	BD
10. D×B	16	890	29	41	21	7
			+	Δ	D	ΔD
11. D×Δ	10	436	30	25	22	22
			+	tr	D	Dtr
12. D×tr	15	701	2	34	62	.8
			+	C	D	DC
13. D×C	13	766	20	23	31	24
			+	tr	V	Vtr
14. V×tr	43	2,847	.6	57	40	.6

+ indicates wild type.

Detailed data from which this table was prepared have been placed on file with "Genetics" and may be consulted by anyone interested.

that the classification of the infertile flies, of necessity, followed the original classification given them. This classification was probably correct in many cases, but the extent of accuracy cannot be known. There were in this group 111 normal, 105 truncate, 9 Curly, and 1 fly classed as wild type which may have been truncate, (it was classed as shrivelled at first but was tested only for Curly). If some of these infertile wild type flies were actually Curly, the Curly class would be larger at the expense of the wild

type class; and likewise, if some of the infertile truncate flies were Curly, the Curly-truncate class would be larger, with a corresponding diminution of the truncate class. The few infertile Curly flies might have been either Curly or Curly-truncate. There were 226 of these questionable flies which comprised 11 percent of the total offspring.

The offspring from 15 pair matings were tested with the results shown in table 1 (1). The total number of flies involved was 2,009, divided approximately as follows: 25 percent wild type, 23 percent truncate, 29 percent Curly, and 22 percent Curly-truncate. The conclusion seems warranted that Curly and truncate are not linked since each pair mating showed a clear division of the progeny into four approximately equal classes, even in the tests which had the greatest number of infertile flies. Since truncate was previously assigned to the first pair of autosomes, on the basis of earlier evidence, Curly can now be regarded as located in the second pair of autosomes (Group III), pending further analysis.

2. *C*×*B*. Turning to table 1 (2), the counts of 35 tests of Curly by Blister gave 1,855 flies of which 37 percent were wild type, 25 percent Blister, 18 percent Curly, and 17 percent Blister-Curly. Since Curly is inconstant, it is evident that a number of flies classed as wild type must have been genetically Curly, and likewise a number classed as Blister were genetically Blister-Curly. This accounts, at least in part, for the size of the wild type and Blister classes as compared with the Curly classes. The concentration of the averages into the wild type and Blister classes does not suggest that the characters are linked, for if they were, the Blister and Curly classes would be expected to be the largest and the wild type and Blister-Curly classes the smallest. This interpretation receives further support from the evidence presented in the following paragraph.

3. *B*×*tr*. Table 1 (3) shows the results of 44 tests of truncate by Blister, involving 3,666 flies. Thirty-four of these were shrivelled; the remainder were approximately 29 percent wild type, 20 percent truncate, 43 percent Blister, and 6 percent Blister-truncate. The most interesting feature here is the excessive number of Blister flies. The probable explanation is that extreme Blister causes a great distortion of the wing so that the whole shape is askew; truncate affects only the shape of the wing; when the two characters are present in the same wing, the presence of Blister so alters the shape of the wing that truncate is concealed. Furthermore, it is the Blister-truncate class which is deficient throughout and the Blister class which is in excess. If some of the flies classed as Blister were actually Blister-truncate, these two groups would tend to become more nearly equal. If the Blister-truncate group represented a crossover class, it would be expected that the wild type class also would be small, which is not the case. Blister is not linked to either truncate or Curly, so tenta-

tively at least, it can be stated that Blister is in the third pair of autosomes (Group IV). Thus each pair of autosomes is represented by a character, and it remains to be shown in which linkage groups the remaining four mutant characters belong.

4. $C \times \Delta$. Delta was studied first in its relation to Curly with the results set forth in table 1 (4). From 29 pair matings 2,727 offspring arose. Twenty nine percent were wild type, 28 percent Delta, 18 percent Curly, and 24 percent Delta-Curly. Here the Curly and Delta-Curly classes are smaller than the wild type and Delta classes. Again it must be emphasized that the inconstancy of Curly obscures the true results. If the Curly gene were present in some of the wild type and some of the Delta flies, as was probably the case, the Curly and Delta-Curly classes would be proportionately increased. This would tend to establish the 1:1:1:1 ratio expected in characters that are not linked.

5. $\Delta \times tr$. Delta was next tested with truncate, as shown in table 1 (5). The 3,985 offspring from 35 pair matings were approxmately as follows: 25 percent wild type, 25 percent truncate, 26 percent Delta, and 22 percent Delta-truncate. Twelve shrivelled flies were not included in the above averages. The flies showing both mutant characters evidently have a lower viability than those of the other classes, for on the average, the Delta-truncate class is the smallest. This is probably not due to any difficulty in classification, for both characters are definite and easily recognized, even when present in the same wing. Truncate and Delta are relatively constant; the percentage of cases in which the characters are concealed is too small to explain the deficiency of the Delta-truncate class on that basis. Furthermore, the three other classes, (wild type, truncate, and Delta) are very nearly equal in size and show no undue concentration of flies in any one class. In three of the matings the progeny were divided into classes almost equal numerically. Evidently Delta and truncate are not linked.

6. $\Delta \times B$. Having found that Delta was not linked either to Curly (Group III) or truncate (Group II) the inference was that it must be linked to Blister (Group IV). The proof of this by crossing Blister with Delta was difficult, chiefly because the two characters are so similar that a double mutant class, if it did arise, would never be distinguishable as such. In addition, the extreme Delta wing so closely resembles Blister that a classification of these groups could never be accurate. A test to secure the information indirectly was made in the following manner. Blister virgin females were crossed to Delta males, the sons of a Delta female. (The reciprocal test was also made.) From individual female progenies arising thus, 26 of the most extreme flies, which might be both Blister and Delta, were outcrossed to wild type males from stock. If any of the females were Blister-Delta, this should be apparent in the progenies. Without exception,

however, there were only two types of offspring from each mating; these were either wild type and Blister or wild type and Delta; never were there three classes. Such results suggest that the two characters can be combined in one fly only with difficulty if at all, and that perhaps they are allelomorphs. This impression is further strengthened by the fact that it is difficult, if not impossible to get either of these characters in a homozygous condition.

7. $B \times F$. Fortunately the linkage of Blister and Delta can be shown through a study of their respective relationship to another character, Fused. The results of crossing Blister with Fused are shown in table 1 (6) The progeny fall into two major classes, Blister and Fused, in almost equal numbers. Of the total offspring from 30 pair matings, 46 percent are Blister and 46 percent Fused. The cross-over classes are exceedingly small, averaging approximately 3 percent wild type and 4 percent Blister-Fused. Not only is the linkage apparent from the averages of all cases, but the same type of result is characteristic of each mating throughout the whole series. In no case is there a grouping of the progeny into four approximately equal classes.

8. $\Delta \times F$. The results of crossing Delta with Fused are similar to those obtained from crossing Blister with Fused. Although the number of tests is less and the averages are smaller, the grouping of the progeny into two major classes is definite, whether viewed from the standpoint of individual matings or of the combined averages. The results are given in table 1(7). From 13 pair matings the progeny are divided as follows: 6 percent wild type, 35 percent Delta, 56 percent Fused, and .9 percent Fused-Delta. In some individual matings the Fused and Delta classes are about equal numerically, but on the whole the Fused class is considerably larger. This probably indicates a poorer viability of Delta.

9. $F \times tr$. That Fused appears to be linked to both Blister and Delta is further supported by the fact that it appears to be independent of both truncate and Curly. The results of the Fused-truncate crosses are given in table 1 (8), the averages for 11 pair matings being 28 percent wild type, 20 percent truncate, 31 percent Fused, and 20 percent Fused-truncate (5 shrivelled flies omitted). In some of these matings the truncate and truncate-Fused classes are smaller than the wild type and Fused classes, which in turn affects the final averages for these groups. Since the results are not typical of linkage (if the characters were linked the truncate and Fused classes would be expected to predominate), it is probable that the poor viability of truncate is responsible. In three of the eleven pair matings, the progeny were divided into four classes of almost equal size.

10. $F \times C$. The final counts of crossing Fused with Curly (table 1 section 9) indicate, when the inconstancy of Curly is considered, that the two

characters are not linked. From 8 pair matings the count was 32 percent wild-type, 15 percent Curly, 34 percent Fused, and 17 percent Fused-Curly. Doubtless some of the wild type flies were genetically Curly and some of the Fused flies were actually Fused-Curly. In summary it may be said that Blister, Delta, and Fused have been shown to be linked and to represent the third pair of autosomes (Group IV).

11. *D*×*B*. Dash was crossed to Blister and to Delta with results essentially alike in both cases, again confirming the linkage of Blister and Delta. In Table 1(10) the averages of 16 crosses of Dash and Blister are: 29 percent wild type, 41 percent Blister, 21 percent Dash, and 7 percent Blister-Dash. The Dash class is much smaller than either the wild type or Blister classes, due probably to poor viability. The small size of the double mutant class would suggest linkage were it not for the fact that the wild type class (which would be the other cross-over class if this were the case) is so large. Probably the flies having two mutant genes were extremely weak and many failed to hatch.

12. *D*×Δ. The results of crossing Delta with Dash are given in table 1(11). Again the two Dash classes are smaller than the wild type and Delta classes but the differences are not as large as in the Dash and Blister crosses. The progeny from 10 pair matings are: 30 percent wild type, 25 percent Delta, 22 percent Dash, and 22 percent Delta-Dash.

Dash could not be tested with Fused because of the similarity of the two characters.

13. *D*×*tr*. The characters Dash and truncate were found to be linked. Table 1(12) shows that there are only two main classes of progeny, truncate and Dash in the backcrosses. The 701 offspring from 15 pair matings are: 34 percent truncate, 62 percent Dash, with the crossover classes represented by only 2 percent wild type and .8 percent Dash-truncate. Two shrivelled flies are not included. On the whole, truncate seems to be less viable than Dash, for there are almost twice as many Dash as truncate flies. The truncate class is deficient in the matings where the total number of progeny is small. In five of the fifteen matings the progenies are fairly large; in these, truncate and Dash are present in about equal numbers. The crossover classes are almost non-existent.

14. *D*×*C*. On the basis of the tests described above (11, 12, and 13) Dash was thought to be located in chromosome Group II. To verify this conclusion it was tested with Curly. It was anticipated that the two characters would be found to be independent of each other, but since a study of the relationship between Dash and Curly would provide additional information concerning the relationship of truncate and Curly (truncate being linked with Dash), the analysis was undertaken in some detail.

The results of the first tests of Dash and Curly are as follows: 13 cases

gave 1 percent shrivelled, 33 percent wild type, 23 percent Curly, 30 percent Dash, and 10 percent Dash-Curly. Since the inconstancy of Curly proved such an obstacle to interpretation of these data, a second test was made in which the non-Curly flies from 5 pair matings were tested further. The initial count was 7 percent shrivelled, 39 percent wild type, 24 percent Curly, 18 percent Dash, and 10 percent Dash-Curly. After testing, however the percentages became: 1 percent shrivelled, 24 percent wild type, 24 percent Curly, 6 percent Dash, and 43 percent Dash-Curly. The infertile flies and the dead flies which could not be tested were tabulated in whatever class they appeared to belong, thus unavoidably introducing some degree of error. This group comprised 13 percent of the total offspring. In the revised count there was an unusually large number of Dash-Curly flies, due probably to the presence of unidentified modifiers which affected Dash to some extent, causing many Dash wings to resemble also the least extreme form of Curly. Since this type of variation frequently occurs and since it was present in pure Dash stock at the time these experiments were conducted, this explanation seems plausible.

The results of the second test were analyzed further, omitting all doubtful flies, and considering only the flies of which the true classification was known. Of 171 wild type flies tested, 42 percent proved to be genetically Curly. Of the 122 Curly flies, 62 percent proved to be Dash-Curly. Correcting the results of the first Dash×Curly experiment on the basis of these percentages, (transferring 42 percent of the wild type class to the Curly class and 62 percent of the Curly class to the Dash-Curly class) the final averages become: 20 percent wild type, 23 percent Curly, 31 percent Dash and 24 percent Dash-Curly. The "corrected" averages are shown table 1(13).

Although there is some inaccuracy in drawing conclusions from such indirect analysis, it seems clear that Curly and Dash are not linked, as the wild type and Dash-Curly flies are too numerous to be considered as crossover classes. These data also further support the conclusion that Curly is not linked to any of the other mutant characters and hence represents the only gene thus far identified in the third pair of chromosomes (III).

15. *V*×*tr*. It is impossible to study the linkage of Varied with any character save the recessive truncate, because Varied has certain features in common with all the others and accurate classification of the progenies would be impossible. Fortunately the tests of Varied with truncate provide all the data needed. As mentioned previously, Varied is not constant; some flies in Varied cultures appear to be wild type when they are genetically Varied. For this reason, all the flies classed as wild type in the Varied-truncate crosses were tested further to verify their classification. In these tests, Varied hatched late in every mating; practically all the truncate

flies hatched before Varied flies began to hatch. This is typical of the Varied cultures in general.

As shown in table 1(14), 43 pair matings were studied. In 23 cases there were no wild type flies to be tested. Of the 53 wild type flies tested more than half were found to be Varied. In the final calculation, among 19 wild type flies, 5 were known to be wild type, 2 were probably wild type but possibly Varied, and the remaining 12 were infertile but were classed as wild type because of their appearance. This whole group formed less than .6 percent of the total progeny. The Varied-truncate group also was exceedingly small, the total number of flies being 18, (less than .6 percent of the total progeny). The flies were mainly of two types, truncate and Varied, the averages being 57 percent truncate and 40 percent Varied. (Only the final averages are shown in table 1). It seems evident from these results that Varied belongs to the truncate-Dash linkage group (II).

Among the tests of wild type females from this series of experiments, four of the matings gave unusual results. These wild type females, heterozygous for truncate, were outcrossed to wild type males from wild stock 2567. The progeny which were expected to include only wild type and Varied contained some truncate flies as well. Further analysis showed that the 2567 stock was contaminated with truncate; the appearance of truncate in these further tests with Varied-truncate was undoubtedly due to this factor rather than to any unusual behavior of the Varied and truncate lines.

SUMMARY

(1) Six new wing characters in *Sciara coprophila* have been studied, all of which are autosomal dominants.

(2) Each character has been shown to segregate selectively in the male so that only characters of maternal origin are transmitted.

(3) A study of linkage has shown that all (except the "limited") chromosomes have now been identified by means of mutant genes and that accordingly the autosomes may be designated as follows:

Chromosome II—truncate, Dash, Varied

Chromosome III—Curly

Chromosome IV—Blister, Delta, Fused

It is not known which pair of chromosomes corresponds to any particular linkage group cytologically, except in the case of the sex chromosome. Since all of the autosomes are paired in the male soma, it is evident that the unpaired chromosome is not an autosome but is the sex chromosome.

(4) The rate of crossing-over for the autosomes was found to be very small, which corresponds to the results obtained previously for the sex chromosomes (METZ and SCHMUCK 1931, METZ and SMITH 1931).

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PREFERENTIAL SEGREGATION IN TRIPLO-IV FEMALES OF DROSOPHILA MELANOGASTER

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INTRODUCTION

THE occurrence of non-random segregation in triplo-IV females of *Drosophila melanogaster* was reported in a preliminary note (STURTEVANT 1934) of which this is the full account. As there shown, and elaborated below, all the tested fourth chromosomes may be arranged in a series, of such a nature that each chromosome will (in a triplo-IV female) pass to the haplo-IV pole at meiosis more often than will any chromosome that lies above it in the seriation. Expressed in another way, if two chromosomes (A and B) are tested against any third chromosome (C), then C will "prefer" that one of the other two that lies higher in the series.

These relations are first described in the case where one of the IV chromosomes concerned carries genes from the X chromosome. The data on other triplo-IV females are then presented. An algebraic analysis that leads to quantitative predictions is then developed.

It should be remembered throughout that the relations discussed are between whole chromosomes, not individual loci—a circumstance made experimentally feasible by the fact that crossing over in this chromosome is negligible in frequency. The chromosomes concerned are designated by the names of the mutant genes they carry, but these genes are to be regarded only as convenient markers, making it possible to follow the behavior of the chromosomes concerned.

DUPLICATION EXPERIMENTS

Males from the stock of scute-10 (=achaete−2) were X-rayed and mated to yellow scute-D1 females. There resulted numerous wild type daughters, and two that showed some of the characteristics of scute. These proved to have two separate modifications of the scute locus added to the achaete of the rayed males; they are designated scute-10−1 and scute-10−2. The latter (scute-10−2) was found to be associated with a reciprocal translocation between the X and IV chromosomes. The break in X lies between the loci of silver and broad (to the right of the so-called "viability gene" of PATTERSON); that in IV lies between the known loci and the spindle attachment. Dr. J. SCHULTZ has examined the salivary glands of this

translocation, and informs me that it is more complex than this, there being a second break in IV beyond the loci of the known genes (the piece of X being intercalated between the two ends of IV). The two new chromosomes (X with left end replaced by IV, left end of X on spindle-attachment of IV) may be obtained separately; the present study is based on flies carrying the shorter of the two as a duplication: that is, diploids plus left end of X on an extra IV spindle attachment. The "normal" X's of such hyperploids carried yellow in the experimental cultures, so that wild type body-color served as an index of the presence of the duplicating fragment.

When such flies (females) were made heterozygous for the "normal" IV chromosomes it was found (STURTEVANT 1934) that the duplication did not segregate at random with respect to these IVs. The two IVs themselves pass to opposite poles in 95 percent or more of the eggs (see below); but the duplication regularly shows a *preference* for one of them. To use the same example as previously cited, in the case of females of the constitution $\frac{y}{y}$ dupl $\frac{ey^D}{gvl}$ crossed with y (not ey^D) ♂, there resulted 673 $+^v\ ey^D$, 372 $+^v\ +^{ev}$, 354 $y\ ey^D$, 806 $y\ +^{ev}$; that is, the duplication passed

TABLE 1

Percentage of cases in which the scute-10-2 duplication went to the same pole as the IV chromosome entered in each column heading, when that chromosome was tested against the ones indicated in the first column. The values entered as probable errors are the probable deviations from 50.0 percent—i.e., $.67.45\sqrt{1/4n}$. The significance of the various symbols, where these are not standard designations for mutant genes, are indicated in the text and in the heading of table 2.

OPPOSED CHROMOSOME	PREFERRED CHROMOSOME								
	$+^{ci}\ ey^R$	*sv*	*ci ey*^{R}	*ey*^{D}	*ci*	X *ci*	*sv*^{ra}	*bl*	*ci*^{D}
$+^{ci}\ ey^R$	50.0		48.5±1.8		48.5±1.4			41.4±1.8	
sv		50.0	47.2±1.3	47.8±1.6	52.2±2.5				43.9±2.4
ci ey^R	51.5±1.8	52.8±1.3	50.0	49.1±1.8	51.1±1.0	47.4±1.2	43.2±0.7	40.4±2.0	
ey^D		52.2±1.6	50.9±1.8	50.0			45.0±1.2	39.2±1.6	39.5±1.5
ci	51.5±1.4	47.8±2.5	48.9±1.0		50.0		46.9±1.0		
X *ci*			52.6±1.2			50.0	48.2±1.3		
+, *y sc w*^{a}			58.7±1.4	49.2±1.9			47.5±1.1		34.7±1.9
sv^{ra}			56.8±0.7	55.0±1.2	53.1±1.0	51.8±1.3	50.0		43.0±1.4
+, San G.			51.9±1.3	61.1±1.8			52.7±2.2		46.3±2.1
+, Bham			60.6±1.6	52.6±1.5			57.6±1.7		51.3±1.3
bl	58.6±1.8		59.6±2.0	60.8±1.6				50.0	44.0±1.2
ci^D		56.1±2.4		60.5±1.5			57.0±1.4	56.0±1.2	50.0
+, Tonto			63.0±1.7	59.0±1.7	53.9±2.0				
bl^D			58.7±1.3	61.7±2.1			56.6±1.7		50.4±1.8
+, Red. M.			68.0±3.1	60.4±1.6			50.6±1.9		51.5±2.6
+. Arr. S.			61.6±2.9	59.0±2.0			63.1±1.4		50.3±1.9
+, Fla.			62.1±1.9	58.4±1.8					
ey		62.4±2.7	59.2±1.2				56.7±1.3	43.7±2.5	60.4±2.7
+, Seattle			61.4±1.8	62.9±1.7			54.9±1.3		
+, Sch. X			64.3±1.3	61.9±1.8					
gvl ey^R			63.8±1.6	65.2±1.4		62.5±1.4	56.0±1.7	55.0±1.4	56.5±1.7
ar	69.1±1.5		70.0±1.3	62.6±2.2			58.0±1.9		59.2±1.8
gvl		65.2±1.7	67.8±0.9	67.0±0.7	60.7±2.6		57.8±1.2	57.0±1.3	55.3±1.4
ey^4		66.4±1.9	66.4±2.2	64.0±2.9			61.3±3.5	57.2±1.7	
M^4							69.4±1.5	60.6±2.7	
ey^2			64.5±1.6	65.8±2.4	65.8±1.7		64.9±1.5	65.8±1.8	62.2±2.5

TABLE 1 (*Continued*)

OPPOSED CHROMOSOME	PREFERRED CHROMOSOME								
	bt^D	*ey*	+, Sch. X	*gvl* ey^R	*ar*	*gvl*	ey^4	M^1	ey^2
$+^{ci}$ ey^R					30.9± 1.5				
sv		37.6± 2.7				34.8± 1.7	33.6± 1.9		
ci ey^R	41.3± 1.3	40.8± 1.2	35.7± 1.3	36.2± 1.6	30.0± 1.3	32.2± 0.9	33.6± 2.2		35.5± 1.6
ey^D	38.3± 2.1		38.1± 1.8	34.8± 1.4	37.4± 2.2	33.0± 0.7	36.0± 2.9		34.2± 2.4
ci						39.3± 2.6			34.2± 1.7
X *ci*				37.5± 1.4					
+, *y sc w*^{a}				43.6± 1.8		45.0± 1.2			
sv^{ma}	43.4± 1.7	43.3± 1.3		44.0± 1.7	42.0± 1.9	42.2± 1.2	38.7± 3.5	30.6± 1.5	35.1± 1.5
+, San G.		45.0± 1.1		42.3± 1.4		42.7± 1.5			31.4± 2.7
+, Bham		44.0± 2.5		43.7± 1.4		42.7± 1.3			39.8± 2.1
bt		56.3± 2.5		45.0± 1.4		43.0± 1.3	42.8± 1.7	39.4± 2.7	34.2± 1.8
ci^D	49.6± 1.8	39.6± 2.7		43.5± 1.7	40.8± 1.8	44.7± 1.4			37.8± 2.5
+, Toronto		49.4± 2.1		47.2± 1.7		42.7± 1.5			
bt^D	50.0	52.1± 1.7		43.2± 1.5		39.0± 2.1			
+, Red. M.		48.9± 2.3		47.8± 1.9		40.7± 1.4			
+. Arr. S.		50.1± 1.6		44.7± 1.7		47.6± 2.2			35.1± 1.9
+, Fla.				42.9± 1.3		50.2± 1.3			
ey	47.9± 1.7	50.0	47.5± 1.5	44.9± 1.1		44.7± 1.1			
+, Seattle				51.1± 2.1		50.6± 2.1			
+, Sch. X		52.5± 1.5	50.0	53.1± 1.4		41.4± 4.4	50.0± 3.7		
gvl ey^R	56.8± 1.5	55.1± 1.1	46.9± 1.4	50.0	46.9± 1.3	47.3± 0.9	41.7± 2.4		43.2± 2.3
ar				53.1± 1.3	50.0	49.5± 1.1	44.7± 1.8		46.5± 1.7
gvl	61.0± 2.1	55.3± 1.1	58.6± 4.4	52.7± 0.9	50.5± 1.1	50.0	50.8± 1.1		51.3± 1.1
ey^4			50.0± 3.7	58.3± 2.4	55.3± 1.8	49.2± 1.1	50.0	53.0± 3.8	
M^1							47.0± 3.8	50.0	47.8± 3.5
ey^2				56.8± 2.3	53.5± 1.7	48.7± 1.1		52.2± 3.5	50.0

to the same pole as the ey^D chromosome in 67.0 percent of the cases. The result was the same whether the duplication was received from the same parent as ey^D (918/1398 = 65.7 percent) or from the same parent as *gvl* (561/807 = 69.5 percent), though in the latter case there resulted more than 50 percent recombination. The result was also the same in attached-X females carrying a Y chromosome (1118/1674 = 65.8 percent) and in separate-X females with no Y (361/531 = 67.9 percent).

TABLE 2

Preference ratios for scute-10-2 duplication in tests concerning a series of wild type IV chromosomes. These came from a mutant stock (yellow^a scute^{12} apricot) and from the following wild stocks: San Gabriel Canyon, California; Birmingham, England; Tonto, Arizona; Redwood Meadow, California; Arroyo Seco, Pasadena, California; St. Augustine, Florida; Seattle, Washington.

OPPOSED CHROMOSOME	PREFERRED CHROMOSOME							
	+, *y sc w*^{a}	+, San G.	+, Bham.	+, Tonto	+, Red. M.	+, Arr. S.	+, Fla.	+, Seattle
ci ey^R	41.3± 1.4	48.1± 1.3	39.4± 1.6	37.0± 1.7	32.0± 3.1	38.4± 2.9	37.9± 1.9	38.6± 1.8
ey^D	50.8± 1.9	38.9± 1.8	47.4± 1.5	41.0± 1.7	39.6± 1.6	41.0± 2.0	41.6± 1.8	37.1± 1.7
ci				46.1± 2.0				
sv^{ma}	52.5± 1.1	47.3± 2.2	42.4± 1.7		49.4± 1.9	36.9± 1.4		45.1± 1.3
ci^D	63.3± 1.9	53.7± 2.1	48.7± 1.3		48.5± 2.6	49.7± 1.9		
ey		55.0± 1.1	56.0± 2.5	50.6± 2.1	51.1± 2.3	49.9± 1.6		
gvl ey^R	56.4± 1.8	57.7± 1.4	56.3± 1.4	52.8± 1.7	52.2± 1.9	55.3± 1.7	57.1± 1.3	48.9± 2.1
gvl	55.0± 1.2	57.3± 1.5	57.3± 1.3	57.3± 1.5	59.3± 1.4	52.4± 2.2	49.8± 1.3	49.4± 2.1
ey^2		68.6± 2.7	60.2± 2.1			64.9± 1.9		

Tables 1 and 2 show the results of the tests of this kind that have been carried out. Examination of these tables will show that the 26 kinds of IV chromosomes that have been tested fall into a series, approximately in the order in which they are listed in the rows of table 1. The nature of this series is such that the duplication "prefers" any chromosome to those that lie below it in the series, and this preference is greater the further apart the two chromosomes concerned lie in the series. This is shown by the fact that the values entered in each column gradually increase from the top to the bottom of the column. There are irregularities in this increase, often great enough to be statistically significant; but the general trend is unmistakable. It is probable that the discrepancies, so far as they lie beyond those to be expected from errors of random sampling, are due to differential viability or (in a few cases) to uncertainties of classification for some of the IV chromosome characters concerned.[1]

The exact seriation shown in the tables is not to be taken as accurate in detail, though the general neighborhood in which each chromosome is placed is clearly correct. The method used in obtaining this sequence, together with a numerical scale for judging the relative degrees of difference concerned, will be described below.

It will be observed that there is no evident relation between the position of a chromosome in the seriation and the mutant genes it carries. The top and the bottom chromosomes are both characterized by carrying recessive eyeless mutants, as is also *ey*, that lies near the middle of the series. Seventh from the top is a wild type chromosome, seventh from the bottom is another; and between these lie several other wild-type chromosomes, at least some of which certainly differ from each other.

Several of the tested chromosomes are derived ones. The first, $+^{ci}ey^{R}$, (from Dr. C. B. BRIDGES), arose by reverse mutation from the third, *ci* ey^{R}. It is probable that these two are really identical in their preference properties. The *gvl* ey^{R} chromosome (also from Dr. BRIDGES) arose by crossing over between *ci* ey^{R} and *gvl;* it is intermediate, definitely different from both, and much more like *gvl*, from which its spindle-attachment is derived. The "+, Sch. X" chromosome was obtained (by Dr. J. SCHULTZ) by crossing over between ey^{D} and *ci*. Here the crossover is much lower in the seriation than either of the chromosomes from which it was derived. Its spindle-attachment comes from ey^{D}, which BRIDGES (1935) has shown to be a compound chromosome, with a diploid laterally attached duplication. This duplication may be important in determining the properties of the ey^{D} chromosome; it is not present in the "+, Sch. X" chromosome.

[1] The position of ey^{D} is different from that reported earlier (STURTEVANT 1934). This is because one of the earlier experiments was carried out with another chromosome ("Scutenick"), not further studied here, that sometimes gives a slight eye defect. These data have been discarded.

The "X *ci*" chromosome arose as a crossover in one of my triplo-IV experiments. The *ci* (and the spindle-attachment) came from *ci ey*^{R}, the +^{ey} either from *ci*^{D} or from *gvl*. In either case the crossover is intermediate but more like the chromosome from which its spindle-attachment came. Evidently there is no one locus responsible for the preference properties of a chromosome, but it is possible that the spindle-attachment end is most important.

The relation of the duplication to the IV chromosomes is evidently due to the fact that it contains IV chromosome materials. A similar (undescribed) duplication, found in the "shaven" stock, carries the loci from *y* to silver, inclusive, but presumably has an X spindle-attachment. Experiments with it, comparable to those in table 1, gave the following values (the numbers given represent percentage preference for the first-named chromosome, which is the higher one in the seriation): *sv* − *ci ey*^{R}, 54.0 ± 3.2; *sv* − *ey*, 46.6 ± 1.9; *ci*^{D} − *ey*, 48.7 ± 2.7; *sv* − *ci*^{D}, 47.5 ± 4.3. These values are clearly not significantly different from 50.0. Similar results were obtained with duplications 107 and 118 (DOBZHANSKY 1934), though here one of the IV chromosomes concerned was a wild type of unknown position in the series.

On the other hand, another duplication derived from an X-IV translocation (an undescribed mottled notch received from DEMEREC) gave clear indications of preference, although the fragment of X concerned is considerably longer than that of scute-10 − 2. The values obtained here were: *ci ey*^{R} − +*y sc w*^{a}, 60.0 ± 2.0; +*y sc w*^{a} − *gvl ey*, 57.4 ± 1.9; +*y sc w*^{a} − *gvl*, 66.9 ± 1.8. These are clearly significant deviations from randomness, in the same direction as the corresponding *sc*$^{10-2}$ values.

TRIPLO-IV FEMALES

When three IV chromosomes are present in a single female, there are three possible types of segregation that give two chromosomes to one pole and one to the other (AB/C, AC/B, BC/A). With the existing material it has not been possible to determine the relative frequencies of all three types of segregation simultaneously in experiments in which contrary classes gave a viability control (except by the use of progeny tests, which have not been carried out). In some of the data the three frequencies were determined (without the viability control), while in others the viability control was obtained but only one of the three frequencies was determined.

Two samples of the raw data are shown in tables 3 and 4. In table 3 appear the results of testing a series of different chromosomes against *ey*^{D} and *ci*^{D}.[2] Here the type of segregation determined is *ey*^{D}*ci*^{D}/x (x indicates

[2] Both *ey*^{D} and *ci*^{D} are fully dominant when present in single dose in triplo-IV flies. It has not been found possible to distinguish such specimens from the corresponding diplo-IVs.

any other chromosome listed in table 3); the viability control is similar to that in a linkage backcross. Contrary classes should be equal; inspection of the table shows that in every case the ey^D character reduced the viability materially. Other experiments indicate that this is not due to the fact that the gene occurs most often in triplo-IV flies, for this condition evidently does not interfere with the viability appreciably—in some cases at least it appears to improve it slightly. The table shows a general tendency for the percentage of tested segregation type to increase as one passes from the top to the bottom of the series of table 1. As in that table, there

TABLE 3

Offspring of triplo-IV females of the constitution $ey^D/ci^D/x$, mated to diplo-IV males not carrying ey^D or ci^D.

x	$ey^D ci^D$	+	ey^D	ci^D	TOTAL	PERCENT $ey^D ci^D/x$
sv	181	242	250	347	1020	41.5
ci ey^R	67	82	163	168	480	31.1
ci	41	57	97	128	323	30.3
+, *y sc* w^a	44	36	49	97	226	35.4
sv^{na}	95	110	170	195	570	36.0
+, SanG.	64	125	111	157	457	41.4
+, Bham	66	84	60	140	350	42.8
bt	127	187	188	270	772	40.7
ey	71	89	77	115	352	45.5
+, Seattle	187	204	232	263	886	44.1
gvl ey^R	129	158	131	179	597	48.1
gvl	144	252	148	238	782	50.6
ey^4	101	137	56	98	392	60.7
ey^2	110	180	77	144	511	56.8

TABLE 4

Offspring of triplo-IV females of the type A/A/B, mated to BB males.

A	B	NOT B	B	TOTAL	PERCENT B
sv	*gvl* ey^R	181	74	255	29.0
sv	*gvl*	577	144	721	20.0
sv	ey^4	174	42	216	19.4
sv	ey^2	202	49	251	19.5
ci ey^R	sv^{na}	633	163	796	20.5
sv^{na}	*ci* ey^R	1227	183	1410	13.0
ci ey^R	*gvl*	791	243	1034	23.5
gvl	*ci* ey^R	771	92	863	10.7
gvl ey^R	*ci* ey^R	185	26	211	12.3
sv^{na}	*gvl* ey^R	976	213	1189	17.9
sv^{na}	*gvl*	1494	415	1909	21.8
sv^{na}	ey^4	1160	389	1549	25.1
ey^4	sv^{na}	659	58	717	8.1
sv^{na}	ey^2	440	155	595	26.0

are exceptions; here the value for *sv* is most aberrant. The explanation of this aberrant value is unknown, though it occurred in several independent tests and appears to be real. The general rule suggested by these results and by those of table 1 is: chromosomes high in the series tend to pass to the diplo-IV pole, those low tend to pass to the haplo-IV pole.

Table 4 shows the results from experiments of the type AAB, where the frequency of B eggs is determined. The frequency of this type of segregation is twice this value; the other two types of segregation are equivalent (AB/A), and their frequency may therefore be calculated ($=\frac{1-2B}{2}$). Here

TABLE 5

Frequencies of types of segregation from triplo-IV females.

FEMALE AND TYPE OF SEGREGATION	NUMBER OFFSPRING	PERCENT	FEMALE AND TYPE OF SEGREGATION	NUMBER OFFSPRING	PERCENT
sv sv/gvl ey^R	255	58.0	*ey^D ci^D/bt*	772	40.7
sv sv/gvl	721	40.0	*ey^D ci^D/ey*	352	45.5
sv sv/ey^4	216	38.9	*ey^D ci^D/+*Seattle	886	44.1
sv sv/ey^2	251	39.0	*ey^D ci^D/gvl ey^R*	597	48.1
sv ey^2/ci ey^R	770	21.6	*ey^D ci^D/gvl*	782	50.6
ey^D ci^D/sv	1020	41.5	*ey^D ci^D/ey^4*	392	60.7
sv gvl/gvl ey^R	276	26.8	*ey^D ci^D/ey^2*	511	56.8
sv ey^2/gvl ey^R	493	23.5	*ci ci^D/ey^4*	249	48.2
sv gvl/ey^2	278	45.0	*sv^{ca} sv^{ca}/gvl ey^R*	1189	35.8
sv ey^2/gvl	278	35.2	*sv^{ca} sv^{ca}/gvl*	1909	43.5
gvl ey^2/sv	—	19.8	*sv^{ca} sv^{ca}/ey^4*	1594	50.2
ci ey^R ci ey^R/sv^{ca}	796	41.0	*sv^{ca} sv^{ca}/ey^2*	595	52.1
ci ey^R ci ey^R/gvl	1034	47.0	*gvl ey^R gvl/sv^{ca}*	573	25.4
ey^D sv^{ca}/ci ey^R	341	28.2	*sv^{ca} gvl/gvl ey^R*	1022	29.5
ey^D ci^D/ci ey^R	480	31.1	*sv^{ca} gvl ey^R/gvl*	—	45.1
sv^{ca} sv^{ca}/ci ey^R	1410	26.0	*gvl ey^R ey^4/sv^{ca}*	338	22.4
ci ey^R bt/sv^{ca}	320	35.6	*sv^{ca} ey^4/gvl ey^R*	361	30.4
sv^{ca} ci^D/ci ey^R	820	23.9	*sv^{ca} gvl ey^R/ey^4*	—	47.2
sv^{ca} bt^D/ci ey^R	280	22.6	*sv^{ca} gvl/ey^4*	398	35.2
ci ey^R sv^{ca}/gvl ey^R	217	36.8	*sv^{ca} ey^4/gvl*	398	32.6
sv^{ca} ey^4/ci ey^R	1074	17.4	*gvl ey^4/sv^{ca}*	—	32.2
ci ey^R ci^D/gvl	831	54.2	*ey^4 ey^4/sv^{ca}*	717	16.2
ci^D gvl/ci ey^R	831	19.1	*ci^D ey/gvl*	247	38.0
ci ey^R gvl/ci^D	—	26.7	*ci^D gvl/ey*	247	33.2
bt^D ey^4/ci ey^R	484	27.3	*ey gvl/ci^D*	—	28.8
gvl ey^R gvl ey^R/ci ey^R	211	24.6	*ci^D gvl/ey^4*	260	43.2
ci ey^R gvl/gvl ey^R	1852	36.1	*ci^D ey^4/gvl*	260	31.4
gvl ey^R gvl/ci ey^R	1110	25.6	*gvl ey^4/ci^D*	—	25.4
ci ey^R gvl ey^R/gvl	—	38.3	*ci^D gvl/ey^2*	763	37.4
gvl gvl/ci ey^R	863	21.4	*ci^D ey^2/gvl*	763	42.2
ey^D ci^D/ci	323	30.3	*gvl ey^2/ci^D*	—	20.4
ey^D ci^D/+y sc w^a	226	35.4	*ey gvl/gvl ey^R*	222	30.6
ey^D ci^D/sv^{ca}	570	36.0	*gvl ey^4/gvl ey^R*	1174	25.2
*ey^D ci^D/+*San G.	457	41.4	*gvl ey^2/gvl ey^R*	1653	27.8
*ey^D ci^D/+*Bham	350	42.8			

there is no viability control, though in every case it may be surmised that the "B" class is slightly decreased from the true value by this factor.

Table 5 summarizes all the data available from triplo-IV experiments (of whatever type) in which as many as 200 flies were recorded. The final column gives the frequencies of the segregation-types concerned (that is, the values of table 3, twice those of table 4). These values have not been corrected, in cases where the viability correction is absent from the experiment itself; but the probability is that such corrections, were it possible to estimate them quantitatively, would be small in most cases.

Many of the values recorded in table 5 are strikingly different from the 33.3 percent that would result from random segregation. Analysis shows that these deviations are in general in agreement with the principle stated in discussing table 3. If A, B, and C represent the three chromosomes concerned, in the order of their position in the seriation, then the three types of segregation occur in the relative frequencies AB/C>AC/B>BC/A. More exact quantitative analysis will be discussed below.

ALGEBRAIC ANALYSIS OF THE DATA

Given three IV chromosomes (A, B, and C), the simplest assumption seems to be that when A and B pass to opposite poles at meiosis, C goes

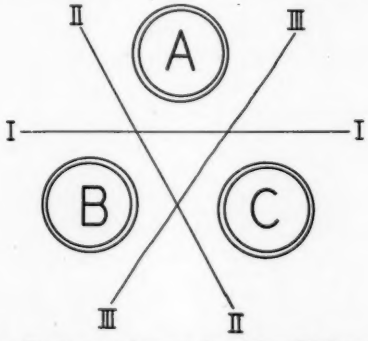

FIGURE 1. Three types of segregation of three IV chromosomes.

to the same pole as A in a fixed proportion of the eggs that is independent of the nature of C. It is clear, from even a cursory examination of the data, that the nature of C does influence the frequency with which A and B separate, so the absolute frequency of AC/B separation will be influenced by the nature of C; the assumption is that the nature of C does not influence the *relative* frequency of AC/B and A/BC.

The testing of this assumption may be approached by an analysis of the three types of segregation into certain possible components. In figure 1 these three possible types are labelled I, II and III.

The terminology used follows:

Let $k = I + II$ (i.e., frequency of separation of A and B)
$l = I + III$ (i.e., frequency of separation of A and C)
$m = II + III$ (i.e., frequency of separation of B and C)

What will hereafter be referred to as the "preference ratios" may then be defined as follows:

r = proportion of k in which C goes with A $\left(\frac{II}{I+II}\right)$

s = proportion of l in which B goes with A $\left(\frac{III}{I+III}\right)$

t = proportion of m in which A goes with B $\left(\frac{III}{II+III}\right)$.

Then

$$I + II + III = 1$$
$$I = l(1-s) = k(1-r)$$
$$II = kr = m(1-t)$$
$$III = ls = mt.$$

These equations may be shown to lead to the following results:

$$I = 1 - \left(\frac{r+s-2rs}{1-rs}\right) \tag{1}$$

$$II = \frac{r-rs}{1-rs} \tag{2}$$

$$III \quad \frac{s-rs}{1-rs} \tag{3}$$

$$t = \frac{s-rs}{r+s-2rs} \tag{4}$$

The first three numbered equations are of interest in dealing with triplo-IV cases; (4) has been used in the analysis of the sc^{10-2} duplication series. The original assumption was that the values of r, s, and t were independent of the nature of the third element concerned—i.e., that r is a constant property of the pair A B, whether they be tested against C, D, or Z. Therefore equation (4) furnishes a method of calculating the preference between

B and C when that between A and B and between A and C is known. The duplication series may be taken as giving such preference values directly, since one type of segregation is rare and does not enter into the calculations. If C (figure 1) be taken as the duplication, the rare type of segregation is III, and the definition of r is that it is equal to II/I+II. However, in most

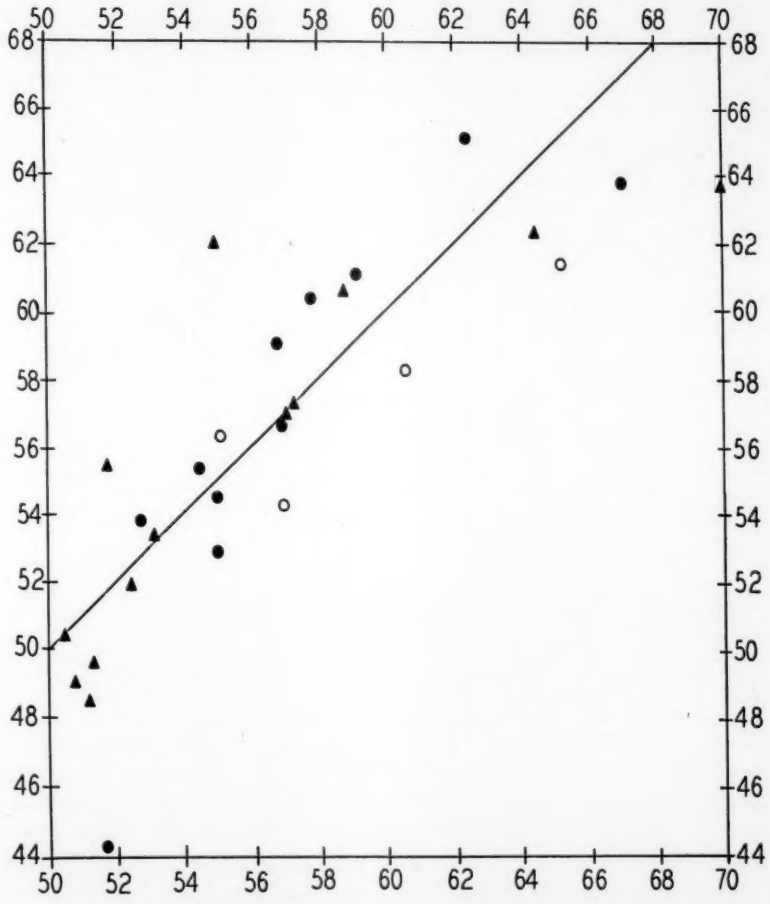

FIGURE 2. Comparison of observed (horizontal axis) and calculated (vertical axis) preference values for the scute-$^{10-2}$ duplication. Solid circles represent cases where the probable error of the observed value is 1.3 or less and the calculated value is an average of 9 or more determinations; solid triangles, cases where the probable error of the observed value is 1.3 or less, the calculated value the average of 5 to 7; open circles, cases where the probable error of the observed value is 1.4 or 1.5, the calculated value the average of 12 or more determinations.

cases the occasional occurrence of III will give rise to two classes, one of which will be indistinguishable from one of those resulting from I or II. Nevertheless, since III is infrequent, the data of tables 1 and 2 may be taken as giving direct determinations of preferences, and equation (4) may be applied to them. (See discussion below.)

Table 6 shows the calculated values of t for each of the 26 tested chromosomes in combination with the five most thoroughly tested chromo-

TABLE 6

Average of calculated preference values (directly observed values also included), based on tables 1 and 2.

OPPOSED CHROMOSOME	PREFERRED CHROMOSOME				
	ci ey^R	*ey^D*	*sv^{ma}*	*gvl ey^R*	*gvl*
+^{ci} *ey^R*	50.4	47.6	41.7	36.7	34.8
sv	48.1	49.6	44.9	36.0	36.4
ci ey^R	50.0	48.6	44.4	36.7	35.3
ey^D	51.4	50.0	45.5	38.1	36.0
ci	51.0	51.0	44.6	40.3	37.8
Xci	53.0	54.8	45.8	39.4	38.7
+*y sc w^a*	55.7	54.3	47.9	40.2	39.2
sv^{ma}	55.6	54.5	50.0	41.2	39.6
+San G.	55.1	56.3	51.1	42.2	40.4
+Bham	57.2	58.5	52.9	44.3	42.7
bt	58.8	56.7	54.7	46.3	43.3
ci^D	59.2	58.4	54.4	45.5	43.7
+Tonto	58.2	60.9	52.1	45.9	45.0
bt^D	60.2	59.5	54.0	45.4	44.5
+Red. M.	59.1	60.4	54.2	46.1	45.5
+Arr. S.	59.9	59.4	55.4	46.7	45.1
+Fla.	60.5	61.3	53.6	46.7	45.3
ey	60.8	60.2	56.9	47.0	44.6
+Seattle	62.9	63.3	56.5	49.7	48.2
+Sch.X	62.4	63.2	57.2	50.2	47.8
gvl ey^R	63.3	61.9	58.8	50.0	47.2
ar	64.6	65.0	60.1	52.6	49.6
gvl	64.7	64.0	60.4	52.8	50.0
ey^4	67.1	66.1	62.2	53.8	50.8
M^4	67.7	67.3	64.0	57.7	51.0
ey^2	68.0	68.4	64.8	55.5	55.1

somes. (See column headings.) From tables 1 and 2 were extracted all cases in which pairs of chromosomes entered in the cells of table 6 were each tested against a common third chromosome. From such combinations t was calculated (for example, *sv*/*ey^D* gave 52.2, *ci ey^R*/*ey^D* gave 50.9; *sv*/*ci ey^R* may be calculated from equation (4), substituting 52.2 for r, 50.9 for s). The various possible calculated values for each cell of table 6 have been averaged; averages of this type have been used directly in figure 2, but in table 6 the observed values from tables 1 and 2 have been averaged in, each being given the same weight as a single calculated value.

The values in this table are clearly self-consistent, each of the columns giving a regularly increasing series of values as one reads from top to bottom. The discrepancies that exist are all small in amount. In effect this table represents a "smoothing" of the raw data of tables 1 and 2. The regularity of the gradation of values is in part inevitable, since the final seriation is based on these data; the essential point is that it is possible to get so nearly a perfect gradation for all five columns simultaneously.

When either r or s is close to 50, equation (4) is approximated by the equation

$$t = 50 + r - s. \qquad (5)$$

That is, t differs from 50 by an amount equal to r − s. This relation is useful in checking calculations; and has also been made use of in applying arbitrary numerical values to the various chromosomes in table 7. This table in appearance, in method of construction, and in practical application, has a resemblance to the familiar "chromosome maps" that are based on crossover values. It is necessary to keep in mind, however, that no spatial relations are represented, and that the table concerns properties of whole chromosomes, not of loci.

TABLE 7.

Preference table (Description in the text).

$+^{ci}ey^R$	20.6	+Bham.	11.2	+Seattle	6.3
sv	19.8+	*bl*	10.4	+Sch. X	6.2+
ci ey^R	19.8	**ci^D**	10.1	**gvl ey^R**	6.2
ey^D	18.6	+Tonto	9.9	*ar*	4.2+
ci	17.4	bl^D	9.6	**gvl**	4.2
Xci	16.2	+Red. M.	9.3	ey^4	2.5
+y sc w^a	14.9	+Arr. S.	9.0	M^4	1.0
sv^{na}	14.2	+Fla.	8.8	**ey^2**	0.0
+San G.	13.5	**ey**	8.4		

The table is based primarily on the relations of the eight most-studied chromosomes; these are represented in bold-faced type. The values of t for each successive pair of these were calculated from the values of table 6—i.e., "second order" calculations were made and averaged. The differences between these values and 50 were then taken as representing the differences between the chromosomes concerned. The interval concerned was first estimated by comparing the totals of the rows in table 6, and assuming the seriation to be such as to give a regularly ascending series of such totals. Each of the remaining 18 chromosomes was then fitted into the scheme by making "second order" calculations (from table 6) for its relations to the two of the chosen 8 better-studied chromosomes between which it lies. When the sum of the values so obtained was not equal to the

difference between the two chosen chromosomes (in no case did it differ markedly) the fitting was done by the method of proportional parts. These calculations resulted in a seriation of the chromosomes, with a set of relative differences. One of the terminal chromosomes (ey^2) was arbitrarily taken as the zero point, and numerical values were assigned to each of the other 25 chromosomes, corresponding to the sum of the differences appearing in each interval between them and the arbitrary zero point.

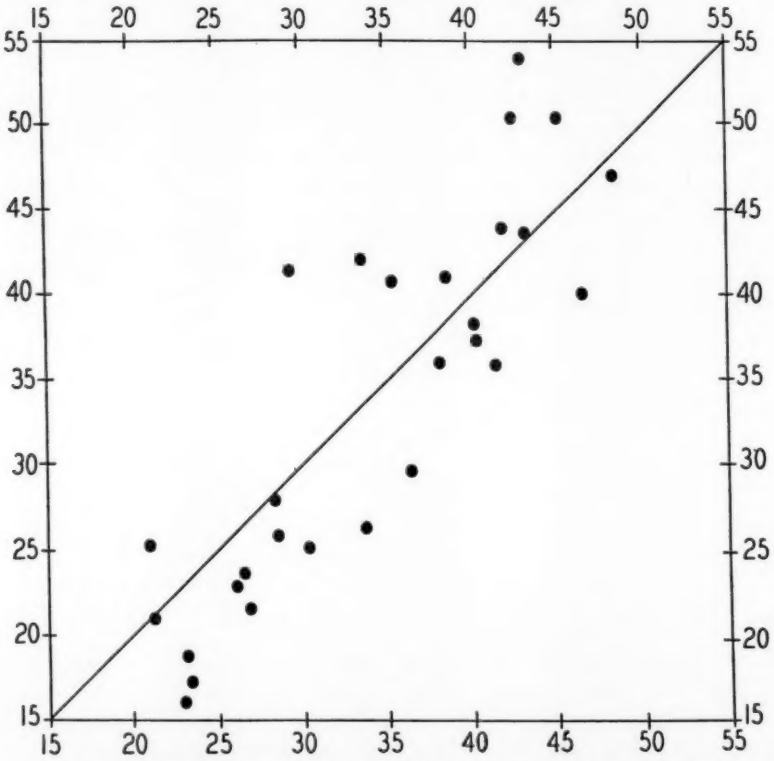

FIGURE 3. Comparison of observed (vertical axis) values for frequencies of segregation types in triplo-IV females with values calculated (horizontal axis) from the scute-$^{10-2}$ duplication experiments. Includes all cases where the observed value is based on 700 or more flies.

Table 7 may be used to give a prediction of the behavior of any three IV chromosomes when tested together. The differences between the values given in the table for any two chromosomes, when added to 50, give the preference ratio for those two. Three such values may be read off (for the possible combinations by two) for any three chromosomes; these, substi-

tuted for r, s, and t in equations (1), (2), and (3), give the required prediction. Furthermore, any IV chromosome not listed in table 7 may be entered in its proper place by means of very few (theoretically by any single one) combinations with known chromosomes; it will then be possible to predict its behavior with any listed chromosomes.

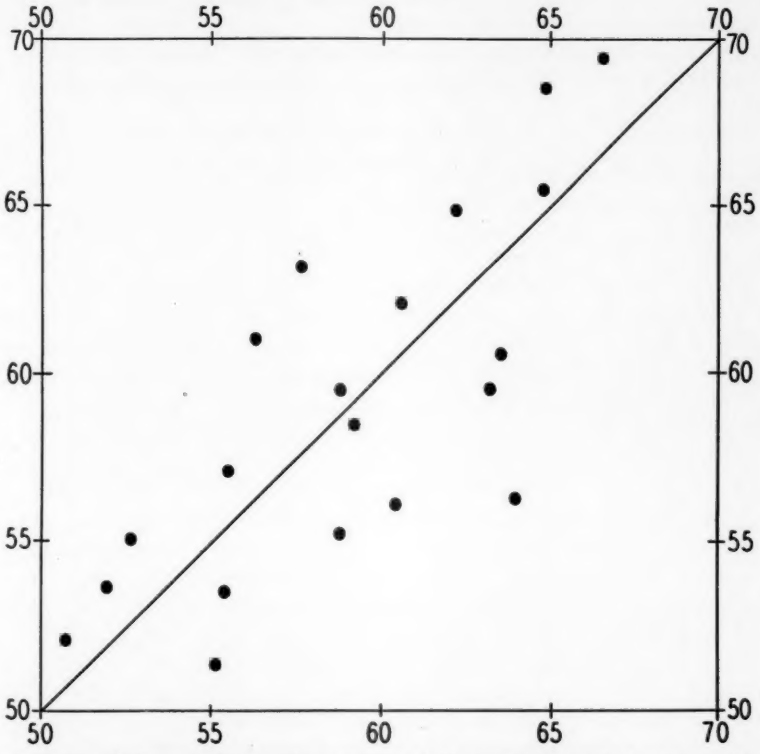

FIGURE 4. Preference ratios calculated from triplo-IV experiments (vertical axis) plotted against those calculated from scute-$^{10-2}$ duplication experiments (horizontal axis).

CORRELATION OF DUPLICATION AND TRIPLO-IV DATA

The equations developed in the preceding section were assumed to apply both to the duplication experiments and to those with triplo-IV females. It is possible to test this directly, by calculating from one series the results to be expected from the other. Figures 3 and 4 show the results of such a procedure.

Equations (1), (2), and (3) make it possible to calculate the types of segregation expected from triplo-IV females, given the preference ratios

of table 6. In some cases the necessary values are not included in table 6; such values were calculated by the same method as that used in deriving table 6. The observed frequencies are recorded in table 5. Figure 3 shows the relations here; all cases where the observed value is based on 700 or more flies are entered. The agreement is evidently satisfactory—calculations based wholly on scute-10—2 duplication data do in fact give good agreement with observations on triplo-IV.

There is, however, a suspicion of a systematic deviation in figure 3, such that observation gives a greater deviation from randomness than cal-

TABLE 8

Preference ratios calculated from triplo-IV data of table 5.

CHROMOSOMES		NUMBER OF DETERMINATIONS	AVERAGE PREFERENCE RATIO
sv	*gvl ey^R*	1	56.1
sv	*gvl*	2	60.5
sv	*ey^2*	1	69.4
ci ey^R	*sv^{na}*	2	57.0
ci ey^R	*ci^D*	1	58.3
ci ey^R	*gvl ey^R*	2	59.5
ci ey^R	*gvl*	4	65.4
sv^{na}	*gvl ey^R*	3	55.1
sv^{na}	*gvl*	3	56.0
sv^{na}	*ey^4*	4	64.8
sv^{na}	*ey^2*	1	68.5
ci^D	*ey*	1	53.5
ci^D	*gvl*	4	61.0
ci^D	*ey^4*	1	63.0
ci^D	*ey^2*	1	62.1
ey	*gvl*	1	53.4
gvl ey^R	*gvl*	2	55.0
gvl ey^R	*ey^4*	2	59.4
gvl	*ey^4*	1	51.9
gvl	*ey^2*	2	51.2

culation would lead one to expect. The problem was therefore approached in another way. From the definitions of r, s, and t it is possible to calculate them from triplo-IV data in cases where the frequencies of all three types of segregation are known. Table 5 includes a number of such cases. The preference values from these were calculated; where several determinations for the same two chromosomes were available these were averaged. The results are shown in table 8, and are plotted against the corresponding values of table 6 in figure 4. Here there is clearly no systematic deviation, so the indication of one in figure 3 is probably not significant. It may be noted that, if a table analogous to table 6 is constructed from the data of table 8, the same seriation of the nine chromosomes concerned will be

found to fit, with surprisingly few discrepancies and these of small magnitude. If the seriation is deduced solely from the data of table 8, it is found to agree with that shown in table 7, with the exceptions that *sv* might be placed below *ci ey*^{R}, *ey* below *gvl ey*^{R} (only one comparison possible), and *ey*^{4} below *ey*^{2}. In all these cases table 7 indicates only slight differences; the rearrangements suggested by the triplo-IV data would not seriously disturb them.

The calculations from the two series thus agree quantitatively as well as could be expected from the nature of the data.

INFLUENCE OF DUPLICATION ON SEGREGATION OF FOURTH CHROMOSOMES

It has been assumed, up to this point, that in diplo-IV females carrying the scute 10 – 2 duplication the frequency with which the two IVs go to one pole and the duplication goes to the other is negligible. It was clear, when the experiments of table 1 were carried out, that this type of segregation does occur; since these experiments regularly gave a few haplo-IV flies (more than the occasional ones found in any experiment), and such flies were always not-yellow (that is, carried the duplication). These flies were recorded, but have not seemed worth dealing with, since the viability of haplo-IV is extremely low and is also quite variable. In one of the experiments of table 1, however, it is possible to identify the other (triplo-IV) product of such segregation, namely in the combination *ey*^{D}/*ci*^{D}/dupl. From this combination there were recorded 146 *ey*^{D}, 106 *ci*^{D}, 94 *y ey*^{D}, 160 *y ci*^{D}, 11 *y ci*^{D}*ey*^{D}. The latter class results from the type of segregation under discussion; an equal number of (not-yellow) haplo-IV must have been produced but failed to survive. From these data one may deduce that the three types of segregation occurred with the following frequencies: *ey*^{D} dupl/*ci*^{D} = 57.9; *ci*^{D} dupl/*ey*^{D} = 37.8; *ey*^{D} *ci*^{D}/dupl = 4.2. It should be noted that the experiment suffers from the low viability of *ey*^{D} referred to before.

Several experiments were carried out in an attempt to determine the frequency of this type of segregation in another way. Females of the type studied in table 1 were mated to males of the constitution *ey*^{D}/*M*^{4}. *M*^{4} is completely suppressed in triplo-IV flies; accordingly any wild type flies must have arisen by the production of a diplo-IV egg by the mother. Since both *ey*^{D} and *M*^{4} have reduced viability, these experiments give maximum values, the haplo-IV eggs always giving less viable zygotes than the diplo-IV ones. The procedure adopted has been to double the number of + zygotes (since a corresponding number of haplo-IVs are lost), and add this number to that one of the two diplo-IV classes that was largest, in order to obtain a total number of zygotes. The data are given in table 9. The average value from this table is 4.55 percent non-disjunction, none

having been found in the few offspring studied from females with no duplication. It may be noted that the highest frequency of non-disjunction was obtained from the combination involving the highest members of the IV chromosome seriation, as might have been expected. Taking this and the ey^D/ci^D/dupl experiment together, we may conclude that 4 percent represents a fair estimate of the usual frequency of this type of segregation.

If 4 percent is taken as the frequency of AB/dupl segregation, it is possible to estimate the error introduced by its occurrence in the experiments of tables 1 and 2. If A and B each carry a recessive gene (a, b), the calculated preference will differ according as the male used in the test is

TABLE 9

Diplo-IV females × ey^D/M^4 males.

CONSTITUTION OF MOTHER	ey^D	M^4	+	CALCULATED NON-DISJUNCTION
sv/ey^4/dupl	90	67	1	2.2
+, *y sc* w^a/sv^{na}/dupl	129	99	6	8.5
sv^{na}/sv^{na}/dupl	204	231	7	5.7
sv^{na}/bt/dupl	102	100	3	5.6
+, SanG/ey^2/dupl	90	67	1	2.2
gvl/*gvl*/dupl	188	160	3*	3.1
Average	—	—	—	4.55
sv/ey^4	191	149	0	0
sv^{na}/sv^{na}	85	80	0	0

* These flies, being *gvl*/*gvl*/M^4, were expected to show the grooveless character, as do *gvl*/M^4 flies. No trace of grooveless was found; the constitution of one of the three flies was verified by tests of its offspring. This result is presumably related to the fact that the grooveless character is often slight in haplo-IV *gvl*.

y a or *y b*. If r represents the true preference ratio (for A over B), then in backcrosses to *y a* males the observed ratio will be r+2/102; in backcrosses to *y b* males it will be r/102. These expressions give the following values:

Male	r=50	r=60	r=65	r=70
y a	51.0	60.8	65.7	70.6
y b	49.0	58.8	63.7	68.6

Table 10 shows the results from all experiments in which females of a given constitution were mated to both kinds of males. The average difference between the two types of experiments, instead of being 2.0 as expected, is only 0.2; evidently this source of error in tables 1 and 2 is too slight to be further considered.

POSITION OF THE DUPLICATION IN THE SERIATION

It is clear that the duplication may be placed in the same seriation as the other IV chromosomes. Since, as just shown, it almost always goes to the diplo-IV pole, it belongs at the top of the series, far above the other chromosomes dealt with. There is too much uncertainty about the values to enable one to place it numerically in relation to table 7.

TABLE 10

Females A/B/dupl mated to males y a or y b.

a	*b*	BACKCROSS TO *a*		BACKCROSS TO *b*		*a* MINUS *b*	
		N	CALC. PREFERENCE	N	CALC. PREFERENCE		
ci ey^R	*gvl*	625	67.2	583	68.5		−1.3
ci ey^R	*sv^{na}*	1979	56.0	361	55.1	+0.9	
gvl	*ey^2*	821	48.2	203	50.8		−2.6
gvl	*ey^4*	378	50.2	587	48.6	+1.6	
ey	*gvl*	200	58.0	802	54.6	+3.4	
Xci	*sv^{na}*	505	52.3	216	48.1	+4.2	
sv^{na}	*ey*	149	53.6	532	57.5		−3.9
sv^{na}	*ey^2*	201	64.1	306	65.4		−1.3
sv^{na}	*gvl*	174	58.6	678	57.5	+1.1	
				Average		+0.23	

IV CHROMOSOME TRANSLOCATIONS

Several reciprocal translocations between the IV and the II or III chromosomes have been tested for preference. They have not given consistent results, presumably because in all such cases there are types of gametes that are not recovered (or have greatly reduced viability). Until these complications can be eliminated it does not seem necessary to present what data were obtained.

NO INFLUENCE OF AGE AND TEMPERATURE

Two separate experiments were carried out to test the effects of age of female and of temperature on the preference ratios. In the first case the tested females were *sv/ey^D*, carrying the scute 10−2 duplication. Here controls (25°) and females treated for two days at 27°C immediately after emergence showed no clear differences from the usual value of 52 percent in daily transfer cultures run for 12 days. In the second series the females were *ci^D/gvl*, with the duplication. The temperatures used were 19°, 25°, and 28°; the first and last were applied for three days that included the emergence from the pupae. Again there were no evident deviations from the usual value (55 percent) in daily transfer cultures made for 12 days. These temperatures and ages are evidently without effect on the preference ratios.

PREFERENCE TESTS IN MALES

The results described in the preceding pages have been based entirely on tests of females. The results obtained from tests of males are shown in tables 11 and 12. Table 11, dealing with scute-10—2 duplication males, suggests that there is no preference in the male, none of the values being clearly different from randomness (50.0). However, it is to be noted that the four combinations that give over 66.0 in females all give above 50.0

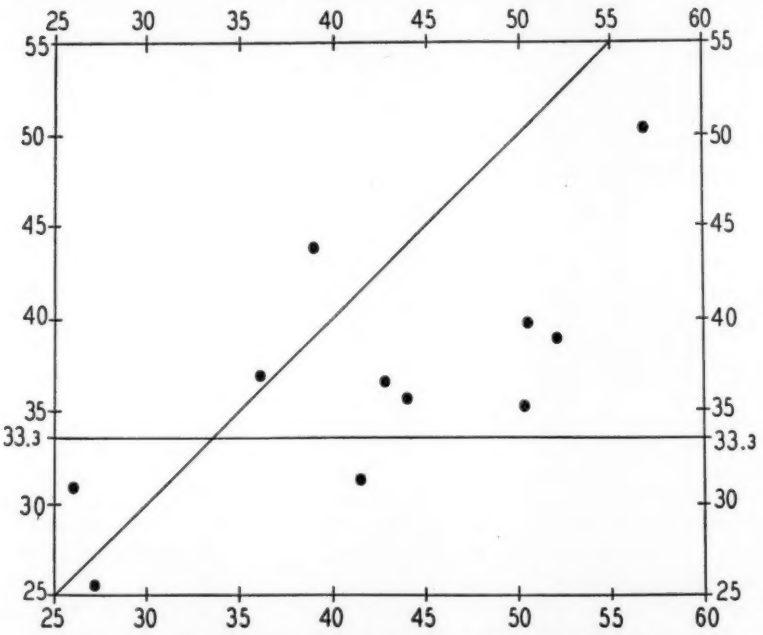

FIGURE 5. Frequencies of segregation types in triplo-IV males (vertical axis) plotted against the corresponding frequencies from triplo-IV females (horizontal axis).

in males, while the four that give 57.0 or less in females all give less than 50.0 in males.

Table 12, showing the results from triplo-IV males, indicates the existence of a preference in males that is less than that in females. The correlation between comparable values for the two sexes is obvious in figure 5. The numbers of combinations are too few to warrant presentation of the results of application of the preference formulae to males; the deviations from randomness are so slight that very large numbers would be required to furnish an adequate test of these formulae.

PREFERENCE OF THE DUPLICATION WITH RESPECT TO X AND Y

Tables 11 and 12 suggest that preference is more marked in triplo-IV males than in diplo-IV males carrying scute-10−2 duplication. One interpretation of this result might be that the unpaired X, having genes in common with the duplication, affects its behavior by reducing its dependence on IV. If this were correct one might expect a relation of the duplication to the X-Y segregation in such males. The available data on this relation follow.

TABLE 11

Preference values for the scute-10−2 duplication in males and in corresponding females.

PREFERRED CHROMOSOME	OPPOSED CHROMOSOME	VALUE OBSERVED MALE	FEMALE
ci ey^R	*ar*	52.7±1.6	70.0±1.3
sv^{na}	*bt*^{D}	49.5±1.4	56.6±1.7
sv^{na}	*ci*^{D}	49.2±2.1	57.0±1.4
ci ey^R	*gvl*	52.6±0.8	67.8±0.9
ey^D	*gvl*	52.0±1.1	67.0±0.7
ci ey^R	*sv*^{na}	49.7±1.7	56.8±0.7
ey^D	*sv*^{na}	46.3±2.1	55.0±1.2
sv	*ey*^{4}	50.8±1.9	66.4±1.9

TABLE 12

Frequencies of segregation types in triplo-IV males and females compared.

	MALES		FEMALES	
	OFFSPRING	PERCENT	OFFSPRING	PERCENT
sv sv/ey^2	488	43.8	251	39.0
ey^D *ci*^{D}*/sv*	346	31.3	1020	41.5
bt^D *ey*^{4}*/ci ey*^{R}	343	25.6	484	27.3
sv^{na} *sv*^{na}*/ey*^{4}	300	35.4	1594	50.2
ey^D *ci*^{D}*/sv*^{na}	597	36.8	570	36.0
ey^D *ci*^{D}/+Bham.	382	36.7	350	42.8
ey^D *ci*^{D}/+Seattle	639	35.7	886	44.1
ey^D *ci*^{D}*/ey*^{2}	253	50.5	511	56.8
ey^D *ci*^{D}*/gvl*	384	39.7	782	50.6
sv^{na} *sv*^{na}*/ci ey*^{R}	592	31.1	1410	26.0
sv^{na} *sv*^{na}*/ey*^{2}	704	38.9	595	52.1

Male *y*, carrying scute-10−2 dupl

	+^{v}♀	*y*♀	+^{v}♂	*y*♂	Total
♀ *yy* (X's separate)	854	1010	772	1031	3667
♀ *ȳȳ* (X's attached)	252	350	314	368	1284

Analysis shows that in both series the duplication more often goes to the same pole as the Y (51.4 ± 0.6 percent and 51.7 ± 0.9 for the two series, 51.5 ± 0.48 for the two taken together). The deviation from randomness is very slight, and is doubtfully significant; since it is in the same direction in both series I am inclined to suppose that it is real.

Nevertheless the suggestion that such a relation is responsible for the difference between triplo-IV and duplication males cannot be taken as verified; for there is a more marked relation of the duplication to the sex-chromosomes in attached-X females, where its preference ratios for IV are unaffected. The data on this relation are as follows: $\bar{y}\bar{y}$ dupl ♀ crossed with y♂ gave + ♀ 1914, y ♀ 1793, + ♂ 1872, y ♂ 2162; preference of duplication for $\overline{XX}$ (as opposed to Y) = 4076/7741 = 52.6 ± 0.38 percent. That the result is not due to viability complications is shown by two facts. First, similar experiments using females with separate X's gave the same four classes as above in the frequencies 1099; 1178; 939; 1076. Here there can be no question of preference, since the mothers carried the duplication, whereas the fathers were responsible for the sex of the offspring. The percentage corresponding to the preference ratio above must then be 50.0 except for deviations due to viability. It is 50.6 ± 0.52. Second, other duplications, that have X spindle-attachments, similar in gene-content to the scute-10–2 duplication, give similar preferences for $\overline{XX}$ as opposed to Y. My own observations on duplication 118 give 307/555 = 56.4 ± 1.4; the data published by DOBZHANSKY (1934, p. 149) show similar relations for duplications 101, 106, 107, 118 and 136. The interpretation of these relations awaits further experiments.

DISCUSSION

The algebraic analysis of the data from triplo-IV females given above has been tested and shown to be adequate. However, the IV chromosomes concerned are cytologically visible bodies, and a satisfactory description of their behavior must have a geometrical basis.

In the earlier account (STURTEVANT 1934) it was indicated that 66.7 was the limiting preference ratio, and that an interpretation might be approached from this observation as a starting point. The more extensive data here presented do not bear out the suggestion of such a limiting value; tables 1 and 6 suggest rather that the value is slightly exceeded and that there is no approach to a limit. If the duplication itself be considered as a IV chromosome, then the preference ratio for it as compared to any other IV chromosome is nearer 90 than 66.7.

One natural assumption is that two of the IV chromosomes pair and pass to opposite poles, leaving the third to go to either pole at random. The preference seriation would then be an indication of the strength of

pairing affinity. This hypothesis alone is inadequate; for if, in *ABC*, pairing is always between *A* and *B*, there will result two types of segregation in equal numbers, and this value of 50 percent is the maximum that can be obtained. Since 50 percent is clearly exceeded in most of the duplication experiments and in several of those with triplo-IV, this hypothesis alone is inadequate.

Perhaps the most attractive of the remaining possible hypotheses is that all three chromosomes conjugate, as in figure 1, and preference is due to non-random orientation of the resulting hexad on the first meiotic spindle. One may suppose that the orientation is always such that one chromosome is directed toward one pole of the spindle, two toward the other. On this basis, a chromosome near the bottom of the preference seriation is one that is more likely to be directed toward a pole; it may be looked upon as being in some sense the equivalent of more than a single one of any type of chromosome lying above it in the seriation. It is probable that a workable model of this type could be imagined in terms of electrical charges.

The scute-10—2 duplication probably has nothing in common with the other IV chromosomes except its inert material and its spindle-attachment. The data on crossover IV chromosomes (of which more are needed) suggest also that these regions of normal IVs may be especially important in determining preference properties. Since the segregation relations of X and Y are also related to inert material and spindle-attachments, it seems likely that they are essential in the relations described in this paper. The spindle-attachment alone cannot be solely responsible, since the crossovers show that more than one locus must be involved. Since BRIDGES (1935) has shown that there is inert material at both ends of the IV chromosome, this demonstration does not weaken the argument for its importance.

SUMMARY

1. Segregation in triplo-IV females of *Drosophila melanogaster* usually gives two chromosomes to one pole, one to the other.

2. The three resulting types of segregation do not usually occur with equal frequencies.

3. The IV chromosomes studied may be arranged in a definite seriation, such that any chromosome will, in a triplo-IV female, pass to the haplo-IV pole more often than will any chromosome that lies above it in the seriation (when the two are tested in like experiments or in the same experiment).

4. If two chromosomes, *A* and *B*, are tested against any third chromosome, *C*, then in those cases in which *A* and *B* separate, *C* will go with *A*

in a proportion of the cases, r, that is independent of the nature of *C*—that is, r is a constant property of the pair *AB*.

5. If the preference ratio for *A* as opposed to *B* is called r, that for *A* as opposed to *C* is called s, and that for *B* as opposed to *C* is called t, then

$$t = \frac{s - rs}{r + s - 2rs}.$$

6. From this and related equations it is possible to show that the data form a consistent whole, and to predict the results that will be given by any combination of three tested chromosomes.

7. The segregation in triplo-IV males is also non-random. It deviates from randomness in the same direction as that in corresponding females, but less markedly.

8. More than one locus is concerned with the preference properties of a IV chromosome.

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EFFECTS OF TEMPERATURE ON FERTILIZATION IN HABROBRACON

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INTRODUCTION

IN THE parasitic wasp *Habrobracon juglandis* (Ashm.), when a cross of unrelated stocks is made, males (haploid) come from unfertilized eggs and females (diploid) from fertilized eggs. When the parental stocks are closely related, not only males from unfertilized eggs but also males (diploid) from fertilized eggs appear. That such biparental males should be produced was puzzling until WHITING brought forth his theory of sex determination in the Hymenoptera (1933a, 1933b, 1935).

According to this theory females have the sex chromosomes X and Y, while haploid males have either X or Y and biparental males either two X's or two Y's. One might then expect equal numbers of biparental male and female offspring according to the principle of random fertilization. However the number of biparental males is always much smaller than that of the females. This deviation from equality WHITING has explained in part by differential mortality, in part by differential maturation. When parental stocks are unrelated differential maturation is complete, all zygotes being XY and resulting in females only. Due to the greater viability of females in contrast to that of diploid males, hatchability of eggs and viability of offspring is much greater when parents are unrelated. This greater fecundity together with lack of biparental males, necessitates the supplementary hypothesis of differential maturation.

It has already been shown (WHITING and ANDERSON 1932) that temperature also affects the production of biparental males among progeny from crosses of related stocks. A much higher percentage of males among biparentals was produced when mothers were set at high temperature (30°C) than at low (20°C), while the percentage of biparentals, males and females, among total offspring was decreased.

After BOSTIAN (1934) produced an orange-eyed stock (11-0) closely related by grading to a certain wild-type stock (11) and giving very high percentages of males among the biparentals, it was shown (ANDERSON 1935) that if stock 11 males were reared at different temperatures (20°, 30°, 36°C) even though the stock 11-0 females were kept at constant temperature (30°C), significant differences appeared in percentages both of males among biparentals and of biparentals among total offspring. In this case

the differences must have been due, not to differential viability of heterosyngamic (XY), homeosyngamic (XX and YY) and unfertilized (X and Y) eggs, but to change in percentage of eggs fertilized and of type of fertilization.

EFFECTS OF CULTURE TEMPERATURES

Preliminary experiment. Females of stock 11-o and males of stock 11 were mated at room temperature and the females were then divided into three groups. One group was set at 32°C, a second at 27°C, and a third at 20°C. As usual the females were transferred through vials a, b, c, d and e when larvae appeared. This was every four days for the 32°C and the 27°C groups, but every ten or twelve days for those at 20°C. No transfer was made to a vial e in the low temperature group.

As in our previous experiments, the higher the temperature the greater is the production of males among biparentals while percentage of biparentals is reduced (table 1). In all three groups (table 2) there is a gradual rise of males among biparentals from vials a through b to c and a slight drop from c to d. In the high temperature groups a very significant rise occurs from vials d to e.

TABLE 1

Stock 11-o females by stock 11 males: preliminary experiment. Totals and percentages with standard errors for the different culture temperatures.

GROUP	TEMPERATURES	BIPARENTAL ♀♀	♂♂	IMPATERNATE ♂♂	PERCENTAGE OF MALES AMONG BIPARENTALS	DIFFERENCES		PERCENTAGE OF BIPARENTALS	DIFFERENCES	
A	32°C	703	242	489	25.61± 1.42	A-B	5.44± 1.78	63.75± 1.27	A-B	2.15± 1.62
B	27°C	1112	281	792	20.17± 1.07	B-C	7.62± 1.97	65.90± 1.01	B-C	6.54± 2.14
C	20°C	356	51	155	12.56± 1.64	A-C	13.05± 2.22	72.44± 1.88	A-C	8.69± 2.27

Egg-counting experiment. As a further check on temperature effects, an experiment was undertaken in which eggs were counted according to methods developed by ANNA R. WHITING and by C. H. BOSTIAN. In much of the tedious technical work involved in this research the writer was greatly assisted by Miss Roger Young, to whom he wishes to express his sincere gratitude.

Stocks 11 and 11-o were used and matings were made at room temperature of males and females reared at 30°C. Sister females were mated to the same males, half of the females mated to each male being set at

lower temperature (18°–20°C) and half at higher temperature (32°–33°C). The females were given caterpillars that had been stung by wasps with mutant factors other than orange and the paralyzed caterpillars were also carefully examined and freed from any eggs observed that were laid by the "foster mothers." The females were then set in the incubators.

For the first four days each wasp was given one stung caterpillar each day. On the fifth day each female was given two caterpillars. The number of eggs laid by the females at high temperature was greatly increased by this increased feeding, but the additional caterpillar had no effect at the

TABLE 2

Biparental offspring from stock 11-o females by stock 11 males: preliminary experiment. Summaries and percentages according to culture temperatures and ages of mothers (vials).

TEMPERATURE	VIALS	BIPARENTAL OFFSPRING ♀♀	BIPARENTAL OFFSPRING ♂♂	PERCENTAGE OF MALES AMONG BIPARENTALS
32°C	a	187	56	23.04
	b	252	86	25.44
	c	149	55	26.96
	d	63	23	26.74
	e	52	22	29.80
27°C	a	267	55	17.08
	b	288	62	17.71
	c	299	87	22.52
	d	166	46	21.69
	e	92	31	25.20
20°C	a	112	12	9.64
	b	137	16	10.46
	c	41	9	18.00
	d	66	14	17.50
Totals	a	566	123	17.85
	b	676	164	19.52
	c	489	151	23.59
	d	295	83	21.96
	e	144	53	26.90

lower temperature. An increase to three caterpillars made no noticeable difference in the number of eggs laid at either temperature. Each caterpillar was placed on a glass slide in the desired position and covered by a small dish containing the female wasp. When observations were made the slide was put on a glass stage of a low power binocular microscope under which was placed a mirror so that the eggs on the under surface could be counted by focussing down on the image. In no case was it necessary to move the caterpillar and thus endanger the results by shaking off the eggs. All egg counts were made in rooms held at temperatures to correspond to those of the incubators in which the eggs were being developed.

The eggs and the larvae for the high temperature group were counted every day until pupation occurred. Eclosion at this temperature took place seven and a half days after the wasps were set with the caterpillars.

For the cool temperature group the eggs were counted on the first day after setting and the females were transferred to new caterpillars. At this low temperature the larvae did not begin to appear until the fourth day. In general if the eggs had not developed into larvae by the sixth day, they failed to develop at all. The larvae were then counted on the fifth and sixth days and checks were made on them every other day until eclosion. Eclosion began about the twenty-seventh day.

In the cool temperature material after the seventh or eighth day, there were noticed several larvae which failed to increase in size and soon died. Although such inviable larvae were not found in the high temperature material developing at a rate almost four times as fast, they may nevertheless have been present. The higher percentage of eggs producing larvae and the lower percentage of larvae producing pupae recorded for the low temperature material (table 3) may be thus explained.

TABLE 3

Stock 11-o females by stock 11 males: egg-counting experiment. Totals, fecundity averages and viability percentages with standard errors at different culture temperatures.

Temperatures	32–33°C	18–20°C
Number of females	11	14
Days set	92	284
Eggs	1839	1990
Larvae	1284	1624
Pupae	1048	1227
Biparental females	428	532
Biparental males	152	67
Impaternate males	321	508
Egg production per day	20−	7+
Percentage of eggs producing larvae	69.82±1.07	81.61±0.87
Percentage of larvae producing pupae	81.62±1.78	75.25±1.05
Percentage of pupae producing adults	84.06±1.13	90.59±0.85
Percentage of eggs producing adults	48.98±1.16	55.63±1.11
Percentage of eggs producing females	23.22±0.97	26.73±0.98
Percentage of eggs producing biparental males	8.26±0.72	3.37±0.47
Percentage of eggs producing impaternate males	17.45±0.87	25.53±0.98
Percentage of males among biparentals	26.21±1.83	11.18±1.28
Percentage of biparentals	64.37±1.59	54.11±1.50

In this experiment we find the percentages of males among biparentals at high and at low temperatures to be approximately the same as in our preliminary experiment. The percentages of biparentals are reversed, however, being higher in the high temperature group than in the low. This is probably due to the smaller number of impaternate males surviv-

ing at the higher temperature, percentage of eggs producing impaternate males. The high temperature, being slightly higher than in the preliminary experiment, may have reduced the viability of the males as has been previously shown (WHITING and ANDERSON 1932).

The percentage of eggs developing into biparental adults, both males and females, is but slightly higher (31.48 percent) in the high temperature material than in the low (30.10 percent). Nevertheless the percentage developing into biparental males is almost two and one half times as great. Evidently homeosyngamic fertilization rather than heterosyngamic is favored by higher temperature. If it be supposed that higher temperature is differentially deleterious to the males among the biparentals, then homeosyngamy must be favored even more than the percentages indicate.

WHITING (1935) suggested sex-reversal of the XX or YY combinations into females as an alternative to the hypothesis of differential maturation but did not think this at all probable, nor did he suggest any explanation for such a reversal. SNELL (1935) proposed a multiple chromosome hypothesis according to which XX or YY might develop into females if heterozygous for any other sex-chromosome pair, ZW for example. The facts herewith reported cannot be explained by the multiple chromosome hypothesis since the material used was genetically homogeneous.

SUMMARY

A preliminary experiment with crosses of closely related stocks of Habrobracon showed an increase in males among biparental offspring but a decrease in biparentals as culture temperatures were increased. At all temperatures percentages of males among biparentals changed significantly with increasing age of mothers.

An experiment in which eggs were counted showed that at the higher temperature the percentage of eggs producing impaternate males was decreased. Among biparental offspring males were increased and females correspondingly decreased.

The conclusion is drawn that increased temperature increases mortality of males and increases homeosyngamy (male-producing combinations, X with X or Y with Y) at the expense of heterosyngamy (female-producing, X with Y).

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GEOGRAPHICAL DISTRIBUTION AND CYTOLOGY OF "SEX RATIO" IN DROSOPHILA PSEUDOOBSCURA AND RELATED SPECIES

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INTRODUCTION

A gene has been found in wild populations of several related species of Drosophila, which causes any male which carries it to produce few or no sons. The cytological analysis has shown why this is so, and also bears on several problems in chromosome behavior. The gene is widely distributed, both geographically and taxonomically, and raises a number of problems in population mechanics.

HISTORICAL

In 1922 one of us found (MORGAN, BRIDGES, STURTEVANT 1925) a strain of *Drosophila affinis* in which occasional males produced families consisting almost entirely of females. The data suggested that this peculiarity was due to the nature of the X chromosome carried by such a male, but the strain was lost before a detailed study could be carried out.

GERSHENSON (1928) later found a similar gene in the European *Drosophila obscura*, and made a careful analysis. He showed that the gene is in the X, and that a male carrying it produces very few sons, regardless of the nature of his mate. Females homozygous or heterozygous for the gene give normal (1:1) sex ratios when mated to normal males, and are fully fertile. Mated to males carrying the sex ratio gene they give the same excess of daughters as do normal females in these circumstances. Egg counts showed that the "sex ratio" result is not due to the death of male zygotes, since there is no greater mortality from such cultures than from controls giving a 1:1 sex ratio. The "sex ratio" gene thus has the effect of causing a male that carries it to produce nearly all X sperm, instead of the usual 50 percent.

GERSHENSON found the "sex ratio" gene to be present (heterozygous) in two of nineteen wild females studied. He pointed out that the gene should automatically increase in frequency in any population, since a male that receives it from only one parent transmits it to nearly all his offspring, while in females it is transmitted like any other gene. In fact such an increase does not occur, but the reason for this is unknown.

In 1929 this same gene, or at least a gene with the same properties, was found to be present in wild *Drosophila pseudoobscura* near Pasadena. The

TABLE 1

Tested X chromosomes in wild females (N—normal, S—sex ratio)

LOCALITY	YEAR	N	S
Race A			
British Columbia			
Shuswap Lake	1934	3	0
Arrowhead	1934	4	0
Nakusp	1934	5	0
Kaslo	1934	2	0
Washington			
Metaline Falls	1934	7	0
Idaho & Montana			
Lake Coeur d'Alene	1934	11	0
Bitterroot Mountains	1934	3	0
Oregon			
Days Creek	1933	2	0
Wyoming			
Big Horn Mountains	1935	3	0
South Dakota			
Black Hills	1935	4	0
Nebraska			
Scottsbluff	1935	4	0
California			
Mount Lassen	1934	3	0
Lake Tahoe	1935	2	0
Sequoia Nat. Park	1933	6	0
Santa Lucia Mountains	1934	49	1
Pasadena	1932	2	0
Pasadena	1933	5	3
Pasadena	1935	14	3
Fish Canyon	1930	5	3
Big Bear Lake	1932	4	1
Big Bear Lake	1934	12	0
Corona del Mar	1932	3	1
Henshaw Lake, near San Diego	1934	7	1
Nevada			
Charleston Peak	1935	3	2
Utah			
Cedar City	1934	2	0
Cedar City	1935	4	0
Zion Nat. Park	1934	2	2
Zion Nat. Park	1935	2	2
Bryce Nat. Park	1935	6	0
Northern Arizona			
Grand Canyon	1935	5	0
Flagstaff	1935	4	2
Southern Arizona			
Santa Catalina Mountains	1935	8	4
Santa Rita Mountains	1935	10	2
Chiricahua Mountains	1935	2	4

LOCALITY	YEAR	N	S
Race A			
Colorado (North)			
Aspen	1934	2	0
Estes Park	1935	5	0
University Camp	1935	4	1
Pikes Peak (slope)	1935	5	1
Pikes Peak (tree line)	1935	5	1
Colorado (South) & New Mexico (North)			
Mesa Verde Nat. Park	1935	5	1
San Juan Mountains	1935	6	0
Zuni Mountains	1935	4	2
Taos	1935	3	0
Southern New Mexico			
Pinos Altos Mountains	1935	4	2
Magdalena	1935	3	3
Carizozo	1935	4	2
Mexico			
Otinapa, Durango	1935	1	1
Cuernavaca, Morelos	1935	6	2
Cerro San Jose, Oaxaca	1935	4	0
Race B			
British Columbia			
Quesnel	1934	1	0
150-mile House	1934	1	0
Oregon			
Newport	1933	8	0
Reedsport	1933	13	1
Gold Beach	1933	11	3
Days Creek	1933	4	0
Oregon Caves	1933	4	2
California			
Klamath	1933	14	6
Eureka	1933	12	3
Shelter Cove (Humboldt)	1933	10	2
Mendocino	1933	4	0
Mount Lassen	1934	5	2
Lake Tahoe	1935	1	0
Sequoia Nat. Park	1933	7	3
Santa Lucia Mountains	1934	8	0

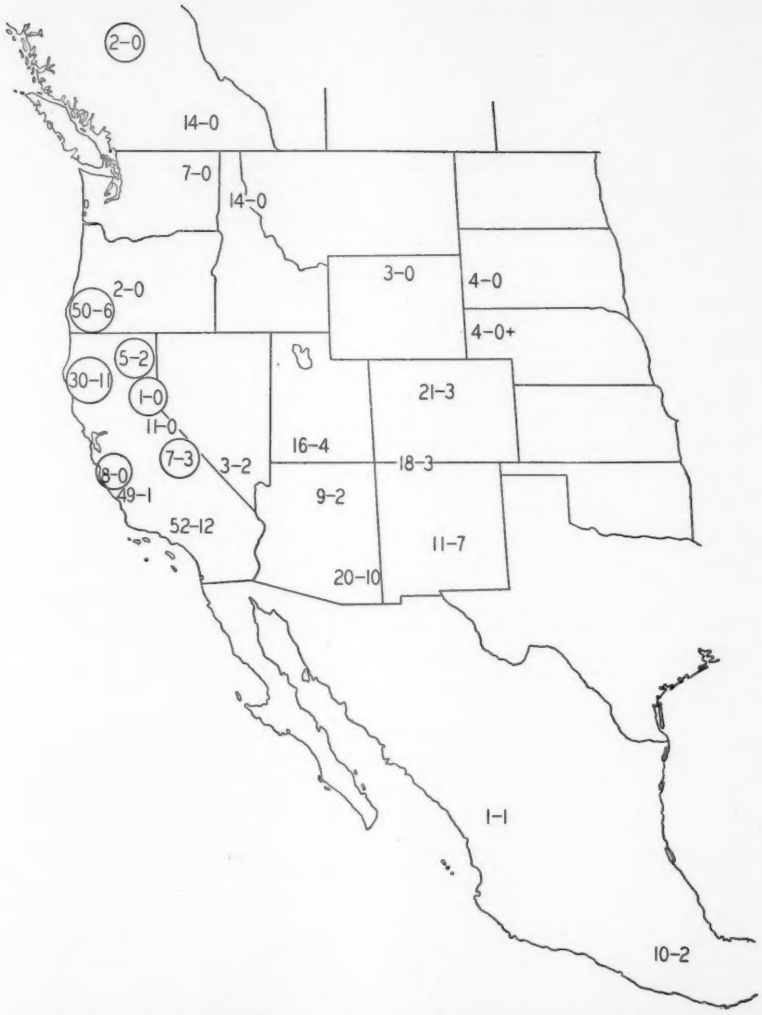


FIGURE 1. Geographical distribution of "sex ratio" factors. In each pair of figures the first indicates the number of tested X chromosomes in which no "sex ratio" was present, and the second shows the number of X's containing "sex ratio." Figures in circles—race B, figures without circles —race A.

present paper gives the results of our study of the geographical distribution and the cytological properties of this form, based chiefly on *D. pseudoobscura*. We have also made additional observations on *Drosophila affinis* and on two undescribed species near *D. affinis* in which "sex ratio" also occurs.

GEOGRAPHICAL DISTRIBUTION OF "SEX RATIO" IN DROSOPHILA PSEUDOOBSCURA

Tests soon showed that the "sex ratio" gene is present in both races (A and B) of *D. pseudoobscura*. Table 1 and figure 1 show its range. These data are based on tests of individual sons of females that were trapped out-of-doors. The attempt was made to test at least five sons from each female studied; in many cases fewer tests were actually made. The following convention was adopted: if both kinds of sons (normal and "sex ratio") were recovered, whatever the total number tested, it was considered that both X's of such a female were tested; if two were tested and were found to be alike it was considered that only one of her X's was tested; if three or more like sons were found it was assumed that both X's of the mother were tested. Other kinds of tests have shown the existence of sex ratio in a few other localities not shown in table 1: one of the wild females collected at Scottsbluff, Nebraska (Race A), and one of those at Days Creek, Oregon (Race B), had mated in nature with a sex ratio male and gave a typical "sex ratio" result. These, together with the records from wild males (table 2) have not been included in the map (figure 1), since they are not directly comparable quantitatively with table 1.

TABLE 2

Tested wild males (N—normal, S—sex ratio)

LOCALITY	YEAR	N	S	LOCALITY	YEAR	N	S
Race A				Santa Lucia Mts.	1934	45	2
California				*Utah*			
Big Bear Lake	1932	9	0	Bryce Nat. Park	1934	1	0
Big Bear Lake	1934	10	0	Cedar City	1934	3	0
Claremont	1932	2	0	Zion Nat. Park	1934	1	1
Fish Canyon	1930	3	1	*Nevada*			
Pasadena	1932	8	3	Las Vegas	1935	3	0
Arroyo Seco	1935	3	0	*Arizona*			
Henshaw Lake	1934	1	1	Gila, near Yuma	1935	0	1
Providence Mts.	1935	1	0	*Race B*			
Upper Kern Valley	1934	1	0	Upper Kern Valley, Calif.	1934	10	0

The map (fig. 1) indicates that the "sex ratio" gene has a wide distribution in both races. In the case of race A it has a maximum frequency near the Mexican border, and decreases to the north, disappearing not far

from the latitude of the northern boundary of California. The detailed data suggest also that the frequency decreases with increase in altitude. In southern California the Big Bear locality (7,000 feet) is much higher than the others (all under 4,000 feet), and has a lower frequency (1/17 as opposed to 11/47). Zion Canyon, Utah (where 4 out of 8 tested chromosomes carried sex ratio) is much lower than the neighboring localities (Cedar City, Bryce National Park, Grand Canyon) from which 17 tested chromosomes were all normal. In both of these cases the tests of wild males agree with those from sons of wild females.

The distribution of "sex ratio" in race B is less clear. There may be an area of maximum frequency on the coast near the Oregon-California line; but more data from other regions are needed.

PROPERTIES OF "SEX RATIO"

Table 3 shows the results obtained from a representative series of "sex ratio" males. It will be observed that the number of males per 100 females ranges from 0 to 17, with an average of about 3 in race A, 8 in race B. The exact numbers are probably not significant, since there are indications that the ratio may be modified by external agents such as tempera-

TABLE 3

Tests of "sex ratio" males (no sex-linked mutants present to produce viability complications)

RACE A				RACE B			
LOCALITY	♀♀	♂♂	♂♂ PER 100 ♀♀	LOCALITY	♀♀	♂♂	♂♂ PER 100 ♀♀
Wild males				*Sons of wild females*			
Zion Canyon	273	0	0	Gold Beach	873	66	8
Henshaw Lake	319	0	0	Oregon Caves	1063	29	3
Gila, near Yuma	303	2	1	Klamath	2584	262	10
Sons of wild females				Eureka	916	54	6
Pikes Peak	297	5	2	Shelter Cove	1334	110	8
Zuni	188	6	3	Lassen	657	74	11
Magdalena	304	4	1	Sequoia	529	58	11
Carizozo	116	20	17				
Pinos Altos	331	4	1				
Santa Catalina	374	26	7				
Flagstaff	460	0	0				
Chiricahua	528	5	1				
Santa Rita	379	6	2				
Zion Canyon	1101	10	1				
Santa Lucia	392	13	3				
Pasadena	239	9	4				
Durango	393	1	0				
Cuernavaca	743	0	0				

ture, and also that it may be influenced by modifying genes. As will be seen from the table, however, there is never any difficulty in determining whether a given male is giving the usual ratio of approximately 100 males per 100 females or the "sex ratio" result.

The sex ratios given by females carrying the gene in question (either homozygous or heterozygous) depend on the male used, and are not different from those given by homozygous normal females. It does not seem necessary to present numerical data to establish this relation.

Preliminary experiments soon established that the "sex ratio" gene is sex-linked, and attempts were then made to locate it with reference to the known sex-linked genes of *pseudoobscura*. Here an unexpected result was obtained: females heterozygous for "sex ratio" were found to give greatly reduced crossing over in the right limb of the X. This result has been obtained with "sex ratio" from several different localities (Santa Lucia, Pasadena, Chiricahua, Pikes Peak, Pinos Altos, Magdalena, Durango in race A; Santa Lucia, Sequoia in race B), and it has been shown (by testing crossovers) that the reduction in crossing over is definitely associated with

TABLE 4

Frequency of crossing over in various crosses involving the standard and the "sex ratio" in race A and B. In each case the frequency of crossing over is shown in percentages, and below is shown the total number of flies on which this percentage is based.

LOCI / CROSS	*sc-y*	*sc-w*	*sc-f*	*y-v*	*y-co*	*w-s*	*v-f*	*v-s*	*f-mg*	*f-s*	*mg-s*	*co-s*
A+/A+				12.6			4.0		20.0		47.0	
				3617			1616		643		251	
A+/A *sr*				13.3			2.1		4.5		0.5	
				1732			1571		920		920	
A+/B+	0.0			8.7	23.8			25.4				4.7
	109			492	235			492				235
A+/B *sr*	0.1				28.2							48.2
	882				882							882
A *sr*/A *sr*				16.5				43.2				
				176				176				
A *sr*/B+		7.4				6.8						
		68				216						
B+/B+			10.0							47.1		
			1109							1109		
B+/B *sr*			13.7							4.4		
			1170							1170		

"sex ratio." Genetic studies of homozygous Pasadena "sex ratio" and cytological studies (in cells of the larval salivary glands) of Santa Lucia and Zion "sex ratio" heterozygotes agree in demonstrating that this reduction is due to the association of "sex ratio" with an inversion of a section of the right limb of the X chromosome, lying not far from the free end of the chromosome. The length of the inversion is equal to approximately one-fifth of the length of this limb, as observed in the salivary gland chromosomes. The only other crossover reducer met with in this chromosome limb genetically, and the only other inversion seen in it cytologically, is that in which races A and B differ from each other (LANCEFIELD 1929, TAN 1935). Accordingly it was desirable to see if race B "sex ratio" is also associated with an inversion, and also how the *sr* sequence of race A is related to the normal race B sequence. This problem has not been studied cytologically; but the genetic data of table 4 show the answer. There are three different sequences: the standard A which is identical with *sr* B; *sr* A; and standard B.

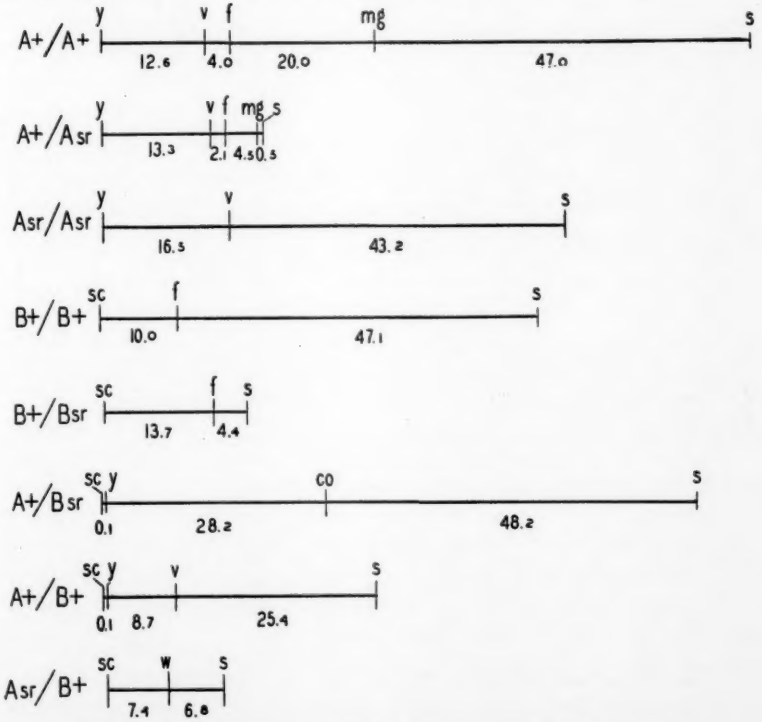


FIGURE 2. Crossing-over frequencies in the X chromosomes in various crosses.

In the case of standard A/*sr* B the actual data obtained are shown in table 5. Contrary classes are not equal in this case, a circumstance that is usual in A-B crosses (DOBZHANSKY and STURTEVANT 1935). However, it is clear that crossovers in both regions 2 and 3 (the latter including the "sex ratio" gene) are viable. None of the classes showed unusual characteristics in excess of the high variability usual in the offspring of A-B backcrosses, so it may safely be assumed that no net duplications or deficiencies were produced, that is, the sequence of genes in the two chromosomes was identical. In general the A-B hybrids show rather more crossing over than is to be expected by comparison with pure A or pure B. This is due to the effects of inversions in other chromosome limbs (SCHULTZ and REDFIELD, in MORGAN, BRIDGES, and SCHULTZ 1932).

TABLE 5

Crossing-over in (A+) / (B *sr*) females	*y* – *sc* (1)	*co* – (2)	*s* – (3)
(+^{sc} classes in left-hand column; male offspring only)			
Non-crossovers	81		245
Singles, Interval 1	1		0
Singles, Interval 2	93		37
Singles, Interval 3	122		184
Doubles, 2 and 3	65		54

TESTS WITH II-Y TRANSLOCATION

SCHULTZ (1933) has reported briefly on a dominant mutant type known as Delta, that was associated with a II-Y translocation. Delta males, mated by normal females, gave mostly normal daughters and Delta sons, plus a few Delta daughters (due to the production of XY sperm) and a few normal sons (XO, due to the production of sperm with neither X nor Y). This translocation was tried against "sex ratio" (Pasadena, A). From normal ♀ × *sr*/Delta ♂ there were produced 1907+ ♀, 166+ ♂, 14 Delta ♂. This result was unexpected, and remained unexplained until the cytological study described below. It is now clear that it was correct in indicating that the Y chromosome is actually absent in most of the sperm of a "sex ratio" male. In the present case the absence of Delta females is even more marked than in the case of +/Delta male, where about 2.6 percent of the daughters were Delta; by comparison at least 49 should have been expected here, whereas none was found.

SPERMATOGENESIS IN "SEX RATIO" MALES

As shown above, the offspring of a male carrying "sex ratio" come from eggs fertilized by spermatozoa possessing an X chromosome but no Y

chromosome. Only a small fraction of the eggs are fertilized by Y-bearing sperm, or by spermatozoa free from either X or Y chromosomes. Since no significant increase of zygotic mortality is observed in sex ratio cultures, the possibility that the eggs fertilized by Y-bearing spermatozoa are inviable is excluded. Hence, the explanation of the behavior of the "sex ratio" may be sought along one of the following two lines. First, the X-bearing and the Y-bearing spermatozoa may be produced in equal numbers, but a majority of the latter may for some reason fail to fertilize the eggs. Second, the spermatogenesis may be so modified that only X-bearing sperm are produced. A cytological study was undertaken with the aim of securing some evidence bearing on this problem. A strain of race A rather than of race B, was selected for this purpose, because in the former the X and Y chromosomes are clearly distinct from each other in meiotic stages, whereas in race B the two chromosomes are more similar.

FIGURE 3. Spermatogonial metaphase plates from males carrying "sex ratio." X=X chromosome, Y=Y chromosome.

Males from one of the "sex ratio" strains were crossed to females homozygous for the sex-linked recessives eosin, magenta, and short. The F_1 females from cultures showing abnormal sex ratios were crossed to normal males, and in the next generation non-magenta males were selected. Since "sex ratio" is closely linked with magenta, these males must carry it. Testes of young males were fixed in Benda's or Navashin's fluid, sectioned 7μ thick, and stained in iron haematoxylin. In the following description the spermatogenesis of the "sex ratio" males is compared with that in normal ones. For the information concerning the latter see the papers by DARLINGTON (1934) and DOBZHANSKY (1934).

The size, shape, and general structure of the testis in "sex ratio" males is normal. The spermatogonia are likewise normal; their resting nuclei have one, less frequently two, nucleoli with one or two satellites. The spermatogonial divisions (fig. 3) show a V-shaped X and a J-shaped Y chromosome, the somatic pairing being apparently as strong as in normal spermatogonia.

The first spermatocytes (fig. 4a) have nuclei with the usual reticulum and several nucleoli of various sizes. Especially in preparations fixed in Navashin's fluid, a careful examination shows that one of these nucleoli,

usually the largest one, is really a precociously condensed chromosome. It is split equationally, and appears as a very short and stout V, or as a broken ring, or as an angular mass. In strongly destained preparations this chromatin nucleolus, which is the Y chromosome, retains the stain longer than other nucleoli (fig. 4a). Since a precocious condensation of the Y chromosome has not been noticed in the normal spermatogenesis, a reexamination of the slides of normal testes has been made. The normal first spermatocytes have from one to several nucleoli; the single, or the largest of the several, appears usually as a regular sphere. In strongly destained or faded preparations the sphere shows a lighter core and a dark

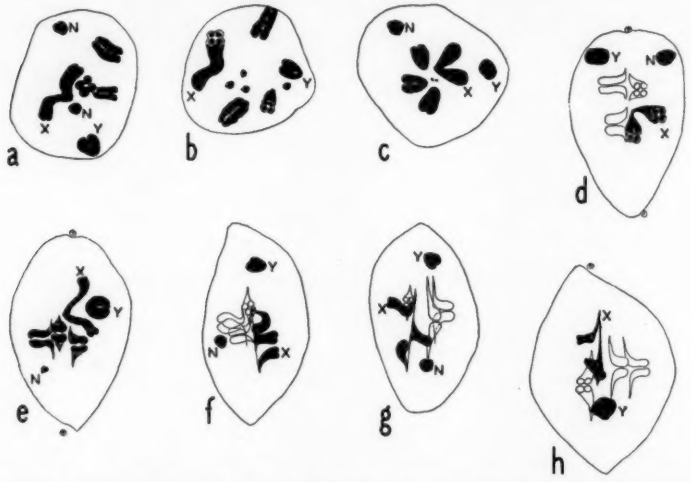


FIGURE 4. Spermatogenesis in "sex ratio" males. a, b—diakinesis; c–h—metaphase and anaphase of the first meiotic division. N—nucleolar fragments; X—X chromosome; Y—Y chromosome. In d, f, g, and h only outlines of the autosomal bivalents are represented.

surface. Only seldom faint indications of doubleness in the nucleolus are noticeable. The relations between this nucleolus and the Y chromosome are uncertain. (Dr. HANS BAUER kindly informs us that in his preparations of the normal testes stained by the Feulgen method the nuclei of the first spermatocytes show no chromatin nucleolus. No satisfactory preparations of "sex ratio" testes stained in Feulgen's have been seen, but here the relation between the nucleolus and the Y chromosome is rather clear. It follows that the precocious condensation of the Y chromosome in the nuclei of the first spermatocytes is the first visible sign of abnormality in the spermatogenesis of "sex ratio" males.)

At diakinesis (figs. 4d, 5a, b) three autosomal bivalents and unpaired X and Y chromosomes become visible. The autosomal bivalents appear in

side view as two rather intimately paired rods, and in end view as four equidistant dots. The appearance and the behavior of the autosomal bivalents at diakinesis and the first meiotic division are normal. The Y chromosome is strongly contracted, but the equational split is usually clearly visible (Y, figs. 5a, b). In early diakinesis (fig. 4d) the autosomes and the X chromosome appear, as usual, first as pale brownish bodies which acquire the full stainability only gradually. The Y chromosome stains much darker; its origin from the "chromatin nucleolus" of the preceding stage can be followed with certainty. The X chromosome, despite

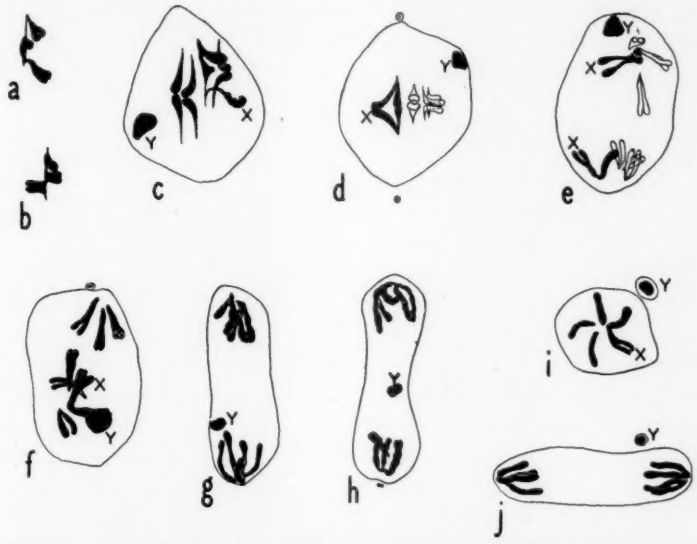


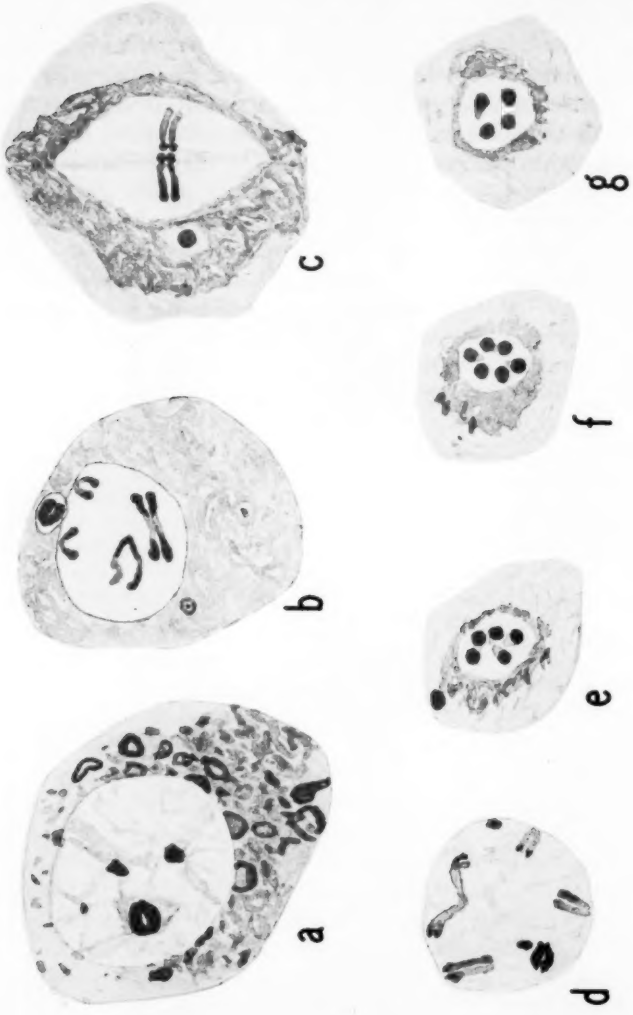
FIGURE 5. Spermatogenesis in "sex ratio" males. a and b—the X chromosome at anaphase; c and d—anaphase of the first meiotic division; e–h—telophase of the same; i—metaphase, and j—telophase of the second meiotic division. X and Y— the X the Y chromosomes respectively.

being an apparent univalent, is uncommonly large and stout (figs. 5a–d). In favorable cells, in which one or both ends of the X chromosome are directed toward the observer, the cause of this is visible: this chromosome has two equational splits instead of the normal one, and is therefore quadripartite instead of bipartite. Whether this quadripartite structure is present in all cells is doubtful, since in some the X chromosome is rather slender (figs. 4d, 5e), but it may be taken for certain that most cells do have quadripartite X's. It is, however, impossible to determine by direct observation how far the double equational split goes in the body of the chromosome (as just stated, it is visible in the ends only). Since in normal

gametogenesis each chromosome is split into two sister strands along its entire length except at the spindle attachment where the two strands are held together, it seems simplest to assume that here too we have four chromatids converging to an undivided spindle attachment. Such an interpretation is in agreement with the observations on the later stages.

At metaphase the autosomes and the X chromosome become arranged in the loose equatorial plate characteristic for the first meiotic division in *Drosophila pseudoobscura* (figs. 5c–h). The Y chromosome takes no part in the equatorial plate and may lie anywhere on the spindle, in the equatorial region as well as in the polar ones. The extraordinary persistence of the nucleolar fragments (N, figs. 5a–g) during the first division seems to be characteristic for the spermatogenesis in the "sex ratio" males. In normal spermatogenesis the nucleolus of the first spermatocytes becomes pale and disintegrates already during the mid-diakinesis, although in some cases the fragments are visible up to metaphase. In the "sex ratio" the fragments are seen even in early anaphase (figs. 5f, g), and some of them are so large and heavily staining that for a time it was suspected that the univalent Y chromosome divides in some cells as early as the first division, which proved to be an error.

At anaphase (fig. 4f–h, fig. 5a–c) the spindle attachment in the quadripartite X chromosome evidently undergoes a division, for the two daughter chromosomes, each showing one equational split, become directed toward opposite poles and away from each other. However, no immediate and complete separation takes place. One of the two arms of each daughter chromosome becomes free, while the other arms are held together for some time. The resulting configurations have a striking resemblance to those observed in the X-Y bivalents of normal *Drosophila pseudoobscura* by DARLINGTON (1934, fig. 20), and attributed by him to the presence of two reciprocal chiasmata in one of the two arms of each chromosome. Whether or not the association of the two X chromosomes in our case is also due to chiasma formation is uncertain. An alternative explanation would be that the second equational split takes place in one arm somewhat later than in the other, and that the unsplit arms are temporarily held together by some force, for instance by an exaggerated somatic pairing. This involves the assumption that the division of chromonemata occurs in a condensed metaphase chromosome. One must also take into account that in some cells, more or less exceptional ones, somewhat different configurations may be seen, as in figure 5d. Here both arms are associated with their homologues at their ends; one of the arms seems single rather than double, although this is not certain. In a few cells (fig. 5f) the division of the spindle attachment does not occur until late anaphase, and the quadripartite X chromosome lags on the spindle.


a
b
c
d
e
f
g

At late anaphase and early telophase (fig. 5e, g, h) three autosomal diads and one X chromosome may be seen passing to each pole. The X is split once equationally, and resembles in all particulars the X seen at one of the two poles in normal spermatocytes. The Y still continues to lag in the spindle; it remains much condensed and its spindle attachment end is not attenuated. The next stage is the division of the cell and the formation of second spermatocytes.

The second spermatocytes (Plate 1b) are normal except for the fact that in some of them besides the normal nucleus also a small micronucleus is found. The micronucleus contains a single chromosome which is evidently the Y chromosome. It follows that during the first meiotic division the Y is not included in either of the two telophase groups, but is left behind, and at the time of the fission of the cell body gets into one of the daughter cells forming there a separate small nucleus. The chromosomes in the second spermatocytes can frequently be counted; all among the sixty-four spermatocytes examined had an X chromosome and no Y chromosomes in their main nuclei. It should be remembered, however, that the "sex ratio" strain used by us for the cytological investigation produces practically only females, and that other strains give rise to some males as well. What happens in the latter is unknown; it is possible that cells like that shown in fig. 4e give rise to one second spermatocyte containing the X and one containing the Y. It is also possible that normal X-Y bivalents occur at the first division in some cells in "sex ratio" males. Some Y-bearing spermatids were found in our strain as well (see below).

The second meiotic division (Plate 1c, figs. 5i, j) is perfectly normal. The chromosomes in the main nucleus form an equatorial plate, and at anaphase the equational halves pass to the opposite poles. The micronuclei containing the Y chromosome take no part in this division. They are not included in the spindle, nor do they form small spindles of their own. The chromosome in the micronucleus becomes small and exceedingly contracted; the equational split in it is no longer visible. It is included in one of the resulting spermatids. At the telophase of the second division the chromosomes in the polar groups can be counted if the spindle is viewed from the pole (Plate 1c–g). Most cells show five dots (Plate 1e), and by focusing it is possible to see that two of the five dots unite at a lower level. These are the two limbs of the X, hence one X and three autosomes are present in such a telophase group. Such counts were made in 200 telo-

DESCRIPTION OF PLATE I

Spermatogenesis in "sex ratio" males. a—first spermatocyte; b—a second spermatocyte with a micronucleus; c—the second meiotic division; d—early diakinesis; e, f, and g—polar views of the telophase groups at the end of the second meiotic division.

phase groups. The results were: 195 groups with X but no Y; 3 groups with a Y but no X (Plate 1g); and 2 groups with one X and one Y (Plate 1 4f.)[1]

The spermatogenesis is normal: some of the young spermatids have a micronucleus with the Y chromosome, but in somewhat later stages the micronuclei are no longer visible. What is their final fate is uncertain; probably they are discarded together with the excess cytoplasm. Cross-sections of the cysts of mature or semi-mature spermatozoa offer the opportunity to count the number of spermatozoa per cyst. The normal number is close to 128 (meiosis occurs in groups of 32 cells, this results in 128 spermatids, DOBZHANSKY 1934). In the "sex ratio" males about four cysts were counted, and the resulting numbers were not far from 128. There is no indication whatever that any spermatids die, or that some of the spermatozoa in a cyst are abnormal.

OCCURRENCE OF "SEX RATIO" IN OTHER SPECIES

As pointed out in the introduction to this paper, "sex ratio" has been reported in *D. affinis* Sturtevant and *D. obscura* Fallén. These two species are rather similar to *D. pseudoobscura* Frolowa; there are also several European and North American species of the same group (mostly undescribed), some of which we have investigated. The European species are being studied by Mr. J. E. COLLIN (Newmarket, England), whose manuscript name *subobscura* is here used for the commonest British species. This is the form whose chromosomes are listed (*fide* C. W. METZ) by MORGAN, BRIDGES and STURTEVANT (1925, p. 182), under the designation "undescribed European species near *D. obscura*." The species here listed as *obscura* Fallén may or may not be the same as that used by GERSHENSON (1928), since the cytological account of FROLOWA and ASTAUROW (1929) indicates that at least two Russian forms occur, both different from METZ's account of *subobscura*. We have in preparation an account of some of the American forms; the species here listed as *athabasca*, *azteca*, and *algonquin* will be described there.

Table 6 shows the data obtained from wild specimens of these various species—data of the same type as those of tables 1 and 2 show for *D. pseudoobscura*.

In the cases of *affinis* and *athabasca* from Woods Hole, and of *azteca* from Cerro San Jose, Oaxaca, Mexico, wild females were found that gave few or no sons. We may assume that these had mated with "sex ratio" males.

"Sex ratio" thus occurs in *pseudoobscura* A, *pseudoobscura* B, *obscura*, *affinis*, *athabasca*, and *azteca;* it was not found in *subobscura* or *algonquin*,

[1] This figure may also be interpreted as resulting from non-disjunction of one of the autosomes; if so an X, four autosomes, and no Y are visible in this group.

but may well be present in them in other localities. The question arises, are we dealing with the same gene in all these cases? That the essential properties are the same is clearly indicated. As shown above, in both races of *pseudoobscura* the gene is located in the right limb of X, is associated with an inversion, and males carrying it give similar frequencies of sons. In *obscura*, GERSHENSON has established all these points except the region occupied in the X and the presence of an inversion. In *affinis* we have found a similar frequency of sons, have shown the gene to be in the X, and salivary gland preparations of heterozygous females show an inversion in the X. In *athabasca* and *azteca* the evidence is less complete, resting chiefly on the sex-ratio itself, though some experiments with *athabasca* are at least consistent with the sex-linkage of the gene concerned.

TABLE 6

Occurrence of "sex ratio" genes in species other than D. pseudoobscura

SPECIES	LOCALITY	YEAR	X'S OF WILD ♀♀		WILD ♂♂	
			+	*sr*	+	*sr*
obscura	Berlin, Germany	1933	2	0	1	0
obscura	Birmingham, England	1932	2	0	3	0
obscura	Newcastle, England	1933	0	0	5	0
obscura	*Moscow, Russia (GERSHENSON 1925)	1925	36	2	0	0
subobscura	Berlin, Germany	1933	0	0	3	0
subobscura	Birmingham, England	1932	17	0	77	0
subobscura	Newcastle, England	1933	19	0	26	0
affinis	Woods Hole, Mass.	1932	4	0	12	1
affinis	Woods Hole, Mass.	1933	3	0	0	0
affinis	Woods Hole, Mass.	1935	12	2	95	8
affinis	Kushla, Ala.	1935	7	0	8	0
algonquin	Woods Hole, Mass.	1932	1	0	1	0
algonquin	Woods Hole, Mass.	1933	2	0	0	0
algonquin	Woods Hole, Mass.	1935	8	0	10	0
algonquin	Mendham, N. J.	1932	2	0	0	0
athabasca	*Woods Hole, Mass.	1935	6	1	1	2
athabasca	Quesnel, B. C.	1934	3	0	0	0
athabasca	Kaslo, B. C.	1934	4	0	0	0
athabasca	Shuswap Lake, B. C.	1934	2	0	0	0
athabasca	Arrowhead, B. C.	1934	2	0	0	0
athabasca	Cape Flattery, Wash.	1934	2	0	0	0
azteca	Cuernavaca, Mexico	1935	5	0	0	0
azteca	Oaxaca, Mexico	1935	2	0	0	0

* Species identification somewhat uncertain.

DISCUSSION

Some items in the spermatogenesis of the "sex ratio" males have a rather general interest. In the first spermatocytes the X chromosome undergoes two equational splits while the rest of the chromosomes split once. This shows that the division of the chromonemata is not necessarily induced by the physiological condition of the nucleus as a whole, but can proceed independently in different chromosomes. This is not new, for in some interspecific hybrids the univalent chromosomes may undergo splitting both at the first and at the second meiotic divisions, while the bivalents split equationally only once. Nevertheless, our case remains unique since here the extra split in the chromosome is known to depend upon the presence of a factor (or a group of factors) localized in a relatively short section of the chromosome involved. The further behavior of the quadripartite X chromosome, and especially the formation of the chiasma-like association between the division products in this chromosome, clearly has a bearing on a number of theoretical problems connected with crossing-over and general chromosome structure. Since, however, we do not consider it established that real chiasmata are here found, a further discussion of this point is premature.

Following DARLINGTON, one might suppose that the reason for nonpairing of the X and Y chromosomes in the "sex ratio" gametogenesis is the extra split in the X chromosome. This, however, helps little in accounting for the subsequent behavior of the X and Y chromosomes. Why, for instance, does the Y chromosome show a precocious condensation and heteropycnosis, and the X behave like the autosomes? Or why is the Y chromosome left in the cytoplasm instead of becoming included in one of the telophase groups? Its univalent condition does not account for either of these phenomena, since the univalents in the hybrids between the A and B races of *Drosophila pseudoobscura* are frequently included in the telophase groups, and if they form separate micronuclei they show signs of activity at the next division (DOBZHANSKY 1934). The only visible difference between the univalents in the hybrids and the univalent Y in the "sex ratio" is that in the former the spindle attachment becomes active after a more or less prolonged delay (as shown by the attenuation of the attachment region), while in the latter no such "activity" is observed. The inherent difficulty of the causal analysis of the behavior of the Y chromosome in the "sex ratio" males lies in the fact that the genetic factor responsible for the whole complex of the deviations in the course of the spermatogenesis is located in the X chromosome only. Despite this, much of the abnormality concerns not the X but the Y chromosome.

"Sex ratio" is associated with an inversion (as compared with the normal X) in both races of *pseudoobscura* and in *affinis* (*obscura*, *athabasca*, and

azteca have not been examined for this point). It seems clear that this association cannot be an accidental one, but there are two reasons for concluding that the inversion as such is not an essential part of the mechanism responsible for the peculiarities of the "sex ratio" X. First, these peculiarities occur only in males which have a single X and are not heterozygous for an inversion, since the region of the X concerned is presumably not one that is in any sense homologous to the Y. Second and more significant, the "sex ratio" sequence of *pseudoobscura* race B is identical with the normal sequence of race A, so that "position effects" resulting from the inversion are excluded. One possible interpretation of the significance of the inversions is suggested below.

GERSHENSON (1928) pointed out that "sex ratio" should automatically increase in frequency in any population containing it, since a heterozygous male transmits the gene to nearly all his offspring, while a female (either homozygous or heterozygous) transmits it in the same frequency as any other gene. Clearly this would be fatal to the race if it did occur; we have, for example, collected a fertilized wild female that gave 236 daughters and one sterile son. It is equally clear that the expected increase in frequency does not occur in nature. Wild populations are somehow kept in equilibrium; but the nature of the counteracting influence can only be surmised. It must be of such a magnitude that it brings about a result equivalent to the production of only about half as many offspring by a "sex ratio" male as by a normal one, on the average. Such an influence should be easy to detect experimentally, but preliminary attempts to locate it have not been successful. These studies are being continued.

GERSHENSON'S point suggests a possible interpretation of the significance of the inversions associated with "sex ratio." If "sex ratio" is in reality not one gene, but two or more complementary ones located in the same general region of the X, then in the absence of an inversion the two would constantly be separated by crossing over and the automatic increase in frequency would be slow. If an inversion occurred in an X that happened to carry both of these hypothetical genes, their separation would be prevented and automatic increase would set in.

The algebraic analysis of populations containing "sex ratio" is difficult, and may best be postponed until more evidence is available concerning the nature of the equilibrium that occurs. These remarks apply even more obviously to the hypothesis of complementary genes just suggested.

SUMMARY

1. "Sex ratio" (symbol *sr*) lies in the right limb of the X of races A and B of *D. pseudoobscura*.

2. Males carrying *sr* give offspring consisting mostly of females, regardless of the nature of their mates.

3. Cytological study shows that, in "sex ratio" males, the X undergoes an equational division at each meiotic division, the Y degenerates, and the autosomes behave normally.

4. The *sr* gene is widely distributed in wild populations of both races of *pseudoobscura*, and what appears to be the same gene occurs also in wild populations of the closely related species *obscura*, *affinis*, *athabasca*, and *azteca*.

5. "Sex ratio" is associated with an inversion in both races of *pseudoobscura* and in *affinis*, the other three species not having been studied.

6. In *pseudoobscura* three sequences occur: *sr* A, normal B, and one that is common to normal A and *sr* B.

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Edited by

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AND

J. B. S. HALDANE

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